INFLUENCE OF LANDSCAPE HETEROGENEITY ON LOCAL AND REGIONAL BIODIVERSITY AND SPECIES COMPOSITION IN NATURAL AND EXPERIMENTAL METACOMMUNITIES

By

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Abstract: Environmental heterogeneity is often studied using differences in resource availability; however, heterogeneity can be represented in a variety of patterns across many types of gradients, which can have diverse effects on local and regional biodiversity. The objective of this dissertation was to identify how environmental heterogeneity gradients influence local and regional biodiversity and species composition in natural and experimental communities. For Chapter 1, I conducted a baseline study in which I surveyed crustacean communities from freshwater and saline wetlands from three study areas, which I refer to as metacommunities, across the Nebraska Sandhills to identify trends in species biodiversity and occurrences in association with local and regional environmental conditions. I found that the alpha richness of freshwater wetlands and metacommunity gamma richness decreased in metacommunities with lower abundances of freshwater wetlands and higher abundances of saline wetlands. For Chapter 2, I surveyed wetland crustacean egg banks from the same systems in Chapter 1, and I found that the egg banks exhibited similar patterns in biodiversity as the emergent crustacean communities surveyed in Chapter 1, and the emergent and egg bank metacommunity structures were strongly associated with the salinity gradient. I hypothesized that the decline in freshwater wetland and metacommunity biodiversity was caused by the decrease in the relative abundance of freshwater habitat patches across the landscape and the increase in the environmental contrast between the freshwater and saline wetlands. I tested this hypothesis in Chapter 3 by using mesocosms to develop experimental metacommunities that were structured along gradients of freshwater habitat abundance and environmental contrast between freshwater and saline patches. I found that my hypothesis was partially supported in that the species richness was significantly lower in freshwater communities and metacommunities from saline-dominated metacommunities. For Chapter 4, I tested a different type of heterogeneity by introducing invasive zebra mussels to experimental metacommunities to generate a disturbance-intensity gradient. I found that dispersal from undisturbed communities in heterogeneous metacommunities were able to mitigate species loss in the disturbed communities. Different sources and levels of environmental heterogeneity will generate different patterns in local and regional biodiversity.

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CHAPTER I

TRENDS IN TEMPORARY POOL WATER CHEMISTRY AND BRANCHIOPOD COMMUNITIES ALONG A LONGITUDINAL CLIMATE GRADIENT IN THE NEBRASKA SANDHILLS

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Abstract

Temporary pools are the most abundant insular aquatic habitats in the world and are ideal systems with which to study ecological and evolutionary processes; however, they are poorly studied in many regions of the world in favor of larger, permanent systems like lakes and rivers. The Nebraska Sandhills, Nebraska, U.S.A. contains thousands of groundwater-fed lakes and temporary pools, ranging from freshwater to hypersaline-alkaline. Much of the variation in water chemistry is associated with a longitudinal climate gradient where the climate is mesic in the eastern Sandhills and transitions to

semi-arid in the western Sandhills. Our objective was to conduct a survey of the temporary pools to learn more about their environmental and ecological characteristics and how they differ along the longitudinal climate gradient. We sampled a total of 30 pools from three areas along the climate gradient from April to July 2013. Branchiopod crustaceans were used to measure ecological characteristics, because they are adapted to live in temporary pools and are sensitive to environmental changes. The frequency and salinity of saline pools increased westwards as aridity increased. Salinity was the primary environmental gradient structuring branchiopod communities. Freshwater alpha and gamma diversity declined westward, which may be due to the westward decline in freshwater habitat. The transition from a homogeneous freshwater-dominated landscape in the eastern Sandhills to a heterogeneous landscape consisting of fresh, low-, and high-salinity waters in the western Sandhills makes this system ideal for studying ecological processes at local and regional scales.

Keywords: Branchiopods, Climate Gradient, Nebraska Sandhills, Salinity, Temporary Pools

Introduction

Temporary pools are biodiversity hotspots across terrestrial landscapes. Due to their high abundance, discrete habitat patches, and wide distributions, they are ideal systems for studying ecological and evolutionary processes (De Meester et al. 2005). Historically, temporary pool research has been neglected in favor of studying larger, more permanent aquatic systems like lakes. However, there has been a large push towards better understanding temporary aquatic habitats, particularly because their small sizes make them vulnerable to anthropogenic disturbance and habitat loss (Schwartz and

Jenkins 2000). The physical, chemical, and biological properties of temporary pools are largely influenced by their geographical landscape; therefore, it is important that these systems are studied from a variety of regions, particularly those that exhibit unique geographical properties.

The Nebraska Sandhills at 50,000 km^2 is the largest grass-stabilized dune system in the western hemisphere and contains water bodies with some of the highest carbonate concentrations in North America. While sand dunes are typically associated with dry desert environments, the interdunal basins yield ~4,000 km^2 of groundwater-fed wetland habitats (i.e., fens, marshes, and temporary pools) and thousands of groundwater-fed permanent and semi-permanent lakes, making it the second most productive waterfowl region in North America (Ginsberg 1985, Gosselin et al. 1999). Most of the aquatic research conducted in this region has been directed towards the lakes with the goal of understanding the mechanisms driving their diverse chemistries. The lakes range from freshwater to hyper-saline/alkaline and contain unusually high levels of potassium and bicarbonates. Most of the saline lakes occur within the semi-arid western Sandhills, where the lakes form in closed basins and evaporation exceeds precipitation, promoting an accumulation of solutes from discharged groundwater (Bennett et al. 2007, Zlotnik et al. 2012). Saline lakes become less saline and less abundant eastwards as the climate becomes more mesic in the eastern Sandhills (Bennet et al. 2007). Very little is known about the ecological and environmental characteristics of the temporary pools of the Nebraska Sandhills, despite their high abundance across the region.

McCarraher (1970) conducted the first survey of Nebraska Sandhills waterbodies in which he sampled hundreds of lakes and temporary pools in search of suitable

waterbodies for fisheries. McCarraher found that, like the lakes, the temporary pools were chemically diverse, ranging from freshwater to saline-alkaline and he suggested that this high chemical diversity contributed to a high regional diversity of anostracan (fairy shrimp) branchiopods, a temporary pool specialist. While McCarraher (1970) collected data from many waterbodies, he only published a small subset of his findings and gave vague site descriptions, making it difficult to resample his sites or use his data for further analysis.

Crustaceans from the class Branchiopoda (anostracans, laevicaudatans, spinicaudatans, notostracans, and cladocerans) are well known for their prevalence in temporary habitats as well as their diverse tolerances to extreme environments such as hypersaline pools, arctic lakes, and desert playas (Brendonck et al 2008). Branchiopods maintain relatively permanent populations in temporary pools through resting eggs, collectively known as the egg bank (Brendonck and DeMeester, 2003). Branchiopod community composition is strongly influenced by the abiotic environment, because their eggs will only hatch when exposed to species-specific environmental cues (i.e., light, temperature, salinity, oxygen, and pH), making them the ideal group for studying species-environmental relationships (Brendonck 1996, Simovich and Hathaway 1997). Furthermore, little is known about what branchiopod species occur within the Nebraska Sandhills.

To address the lack of abiotic and biotic information on the temporary pools of the Nebraska Sandhills, we conducted a survey in which we sampled 30 temporary pools across the Nebraska Sandhills during the spring and summer of 2013. Our objective for this study was to document the environmental characteristics of the temporary pools of

the Nebraska Sandhills and their associated biological communities; we were particularly interested in how the temporary pools differed environmentally and ecologically across the east to west longitudinal climate gradient that overlays the Sandhills region.

Methods

Study Sites

We sampled a total of 30 temporary pools from three study areas (Fig. 1; eastern, n=9; central n=11; and western n=10). Much of the Sandhills region is privately owned for cattle production, so we selected the sites based on public accessibility (i.e., roadside pools and wildlife refuges) or landowner permission. Most of the sites in the eastern Sandhills were roadside pools that were either constrained within ditches or extended into nearby pastures. In the central Sandhills, we targeted pools on the Valentine National Wildlife Refuge. In the western Sandhills, we sampled roadside pools and pools on the Crescent Lake National Wildlife Refuge.

Sampling

We sampled the pools over a two-day period monthly from April through July 2013, encompassing the beginning and ending stages for most of the pools' inundation periods (Van den Broeck et al. 2015). Some of the saline pools still contained water in July; however, the water levels were greatly reduced from previous months and the invertebrate communities were diminishing, so we ceased sampling after July. We used a Horiba Multi-parameter water quality probe to measure salinity (mS/cm), pH, turbidity (NTU), temperature (°C), and dissolved oxygen (mg/L). Chlorophyll *a* samples were

collected in the field by filtering a known volume of water through Whatman GF/F glass fiber filters, which were then wrapped in foil and frozen until analysis. Chlorophyll *a* was extracted from the filters with 90% basic methanol for 20 hours (Clescerl et al. 2005), and was measured using a Trilogy Turner Fluorometer. We collected 1L water samples in brown bottles and placed them on ice until we returned to the laboratory to process them for total phosphorus (TP) and total nitrogen (TN). We measured TP with a spectrophotometer following a persulfate digestion (Clescerl et al. 2005), and TN was measured using a HACH Total Nitrogen persulfate digestion kit and HACH DR 5000 spectrophotometer. Due to unusually high levels of phosphorus in the saline pools, we had to dilute some of the samples with reagent grade water to 1-10%. All of the water quality measurements and water samples were recorded and collected from the shore of the pool, so as not to disturb the sediment at the bottom. We measured the surface area of each pool by fitting a polygon to the shape of the pool in Google Earth Pro (Visser et al. 2014).

We collected branchiopods with a 250 micrometer-mesh dip-net with 3-minute sweeps throughout the water column and available microhabitats. The branchiopod samples were preserved in 95% ethanol in the field and transferred to fresh 95% ethanol after being processed in the laboratory. Branchiopods were identified to species using Goulden (1968), Belk (1975), Martin and Belk (1988), Pennak (1989), Rogers (2001), Thorp and Covich (2010), and Haney et al. (2013), and recorded as present or absent, while other invertebrates were identified to order or family when possible.

Environmental Characteristics

We classified all of the pools that were less than 3.0 mS/cm as freshwater and those 3.0 mS/cm and above as saline *post hoc* based on the physical and community characteristics of the pools described later as well as what has been reported in the literature (Hammer 1986). We performed regression analyses using the seasonal mean of each environmental variable for each site to show how they transitioned along the east-west longitudinal climate gradient in the freshwater and saline pools, as well as, to display the environmental contrasts between freshwater and saline pools along the east-west climate gradient. The seasonal means were derived from the environmental data collected from April to July, which depending on inundation length consisted of three to four data points.

Community-environment relationships

Ordination analyses were conducted independently for each study area to determine which environmental variables were the most important in structuring branchiopod communities within each area. Salinity was log-transformed and turbidity was square root transformed for normality prior to conducting analyses, and we used Pearson correlation matrices on SigmaStat 3.5 to remove highly correlated ($\rho > 0.7$; $p = 0.05$) variables. We performed a partial forward selection Canonical Correspondence Analysis (CCA; 999 Monte Carlo permutations) using CANOCO 5.04 on the community compositions of each region across all four sampling dates. Only environmental variables that significantly explained community variation ($p=0.05$ with Bonferroni correction) were incorporated into the CCA. Temporary pools undergo successional stages, so sampling date is likely to be a strong contributor to changes in community composition (Jocque et

al. 2007); therefore, we used sampling date as a covariate so that we could account for temporal effects on community composition.

Species Diversity

We calculated the alpha (average number of species at each site), gamma (total number of species across all sites), and beta (species dissimilarity between sites) diversities for each study area to determine how branchiopod biodiversity differed across the three study areas. We compared mean alpha diversity between study areas using a one-way ANOVA and the Holm-Sidak method for post-hoc pair-wise interactions. We calculated beta diversity by dividing gamma diversity by mean alpha diversity. We only had single values for beta and gamma diversity for each study area, so we could not compare them statistically. Few species can tolerate saline environments, so when comparing the mean alpha diversity between study areas with and without saline pools, the areas with saline pools will often have a lower mean alpha diversity (Williams 1998). Also, calculating total diversity metrics for each study area could overshadow interesting patterns in biodiversity that may be specific to freshwater or saline pools. Therefore, after calculating the overall alpha, beta, and gamma diversities of each study area, we split the sites into saline (>3.0mS/cm) and freshwater (<3.0mS/cm) groups and calculated the alpha, beta, and gamma diversities for each study area within these two groups. We compared the freshwater alpha diversity between study areas using a one-way ANOVA and the Holm-Sidak method for post-hoc pair-wise interactions. The alpha diversities of the saline pools were non-normal, so we used a Kruskal-Wallis one-way ANOVA on Ranks and Dunn's method for post-hoc pair-wise interactions. We performed regression

analyses using only the environmental variables that explained significant variation in the CCA's above to determine how alpha diversity was influenced by the environment. We used regressions for each study area independently and then across the entire dataset to determine whether the influence of the variables on alpha diversity were study-area specific or a generalized pattern. We then separated the freshwater sites from the saline sites and performed regressions on the segregated datasets to determine whether the environmental-alpha diversity relationships differed between saline and freshwater sites. All of these analyses were conducted in SigmaStat 3.5.

Results

Habitat Characteristics

The freshwater and saline pools were easily distinguished by sight based on water color, the presence/absence of aquatic vegetation, and the presence/absence of evaporite mineral formation along the pool shores. Freshwater pools were often a transparent blue color and were covered with diverse assemblages of aquatic vegetation. The saline pools often had amber-colored water that, depending on sampling date, were either transparent with the entire water column visible or highly turbid. Rushes often grew along the outer-rims of the low salinity pools with no aquatic vegetation growing throughout the pool. The high salinity sites were completely devoid of aquatic vegetation, surrounded by barren sandy shores covered in evaporite minerals. The bottoms of the saline pools were often covered in large algal mats. Saline pools that were near railroad tracks had dark black water. However, despite appearing to be highly polluted, the black pools supported

similar branchiopod communities as the other saline pools, but the shores of the low-salinity black pools did not support aquatic vegetation like the normal low-salinity pools.

Environmental Characteristics

The only environmental variable that significantly increased westwards in both freshwater and saline pools was pH (Fig. 2; pH: freshwater $p < 0.001$; saline $p = 0.009$). Salinity increased significantly westwards in the saline pools (Fig. 2; salinity: freshwater $p = 0.1$; saline $p = 0.04$). Turbidity and TP appeared to increase westwards in saline pools; however, the trend was not statistically significant (Fig. 2: turbidity, $p = 0.334$; TP, $p = 0.30$). Many of the freshwater and all the saline pools were classified as being hypereutrophic with some saline pools reaching unusually high phosphorus levels (>1,000 µg/L) for habitats that do not receive agricultural runoff (see Supplementary Data: Table 1). Salinity, pH, turbidity, and TP were generally higher in saline pools than freshwater pools (Fig. 2). The freshwater pools exhibited very little variation within and between the three study areas across all the environmental variables except for surface area; whereas, saline pools were highly variable both within and between study areas for many of the environmental variables (Fig. 2).

Community-environment relationships

In the eastern Sandhills, the measured environmental variables did not explain community variation (CCA: $p = 0.896$). Salinity and surface area were the only variables that significantly explained community variation in the central and western Sandhills with salinity explaining more variation than surface area (salinity: central = 24.9%,

western = 35.6%; surface area: central = 7.6%, western = 6.3%; Fig. 3). Environmental variables explained more variation overall in the western Sandhills (41.9%) than in the central Sandhills (32.6%) (Fig. 3). *Moina macrocopa* (cladoceran), *Daphnia similis* (cladoceran), *Pleuroxus trigonellus* (cladoceran) and *Branchinecta potassa* (anostracan) were only found in saline pools, and were often the only branchiopods detected in the high salinity pools (Fig. 3, 4). *Ceriodaphnia dubia* (cladoceran), *Daphnia pulex* (cladoceran), *Chydorus sphaericus* (cladoceran), and *Simocephalus vetulus* (cladoceran) were the most common freshwater cladocerans and were found together at 16 of the 19 freshwater sites across all three regions. *Ceriodaphnia dubia* (cladoceran) was the most common species and was detected at all the freshwater sites and at 5 saline sites with low-moderate salinities (<10mS/cm) (Fig. 4). The freshwater large branchiopods *Eubranchipus bundyi* (anostracan), *Eubranchipus ornatus* (anostracan), *Lepidurus couesii* (notostracan), and *Lynceus brachyurus* (laevicaudatan) frequently co-occurred in freshwater pools; however, the group diminished westwards as freshwater habitats declined. *Eubranchipus ornatus* (anostracan) were only detected in the eastern Sandhills, *L. couesii* (notostracan) were detected in the eastern and central Sandhills, and *E. bundyi* (anostracan) and *L. brachyurus* (laevicaudatan) were detected in all three study areas. Of the large branchiopods, *L. brachyurus* (laevicaudatan) was the most widely distributed, occurring in 16 of the 19 freshwater pools, and this is the first record of the order Laevicaudata in the state of Nebraska.

Species Diversity

Overall, we found 34 branchiopod species (Fig. 4; see Supplementary data: Table 2), five of which were large branchiopods. In the combined dataset (both freshwater and saline), alpha diversity was significantly different between each of the three regions (ANOVA, $p < 0.001$; post hoc Holm-Sidak method, $p = 0.05$), being highest in the eastern Sandhills with 13.1 ± 1.02 species and declining westward to 10.71 ± 0.71 and 7.3 ± 1.86 species in the central and western Sandhills, respectively (Fig. 5 a). Both freshwater and saline alpha diversity declined from east to west and were significantly different across regions (ANOVA, freshwater $p = 0.016$; saline $p = 0.006$; Fig. 5 b, c). Neither beta nor gamma diversity were statistically analyzed, because we did not have replication. However, beta diversity appeared to increase from east to west in the combined dataset, decrease from east to west in freshwater pools, and increase from east to west in saline pools (Fig. 5 d, e, f); and gamma diversity appeared to decrease from east to west in all three datasets (combined, freshwater, and saline) (Fig. 5 g, h, i).

There was an overall significant negative relationship between salinity and alpha diversity (Regression: $p < 0.001$, $R^2=0.593$; Table 1). Salinity had a significant negative effect on alpha diversity in the central and western regions (central, $p = 0.040$, $R^2=0.322$; western, $p = 0.048$, $R^2=0.329$, Table 1). Pool surface area did not have an effect on alpha diversity in the combined dataset or on alpha diversity within each individual region (Table 1). Surface area had a significant positive relationship with alpha diversity across the full freshwater pool dataset (Regression: $p = 0.003$, $R^2=0.369$ Table 1). Surface area had a positive relationship with freshwater alpha diversity in the central region, but it did not influence freshwater alpha diversity in the other regions (Table 1). Surface area had an overall negative influence on alpha diversity in the saline pools (Regression: p =

0.047, R^2=0.301; Table 1); however, surface area did not have an influence on saline alpha diversity in the individual regions (Table 1).

Discussion

Here, we conducted the first targeted survey of temporary pools from the Nebraska Sandhills. The Nebraska Sandhills is overlain by a climate gradient in which changes in precipitation and evapotranspiration rates combined with unique geological characteristics (i.e., permeable soils and closed basins) have produced a diverse assemblage of freshwater and saline waterbodies. We observed a westward increase in habitat heterogeneity in which the eastern Sandhills were dominated by freshwater pools, the central Sandhills contained both freshwater and low-salinity pools, and the western Sandhills was dominated by low- and high-salinity pools with few freshwater pools. This pattern in landscape heterogeneity had only been observed in the lakes of the region (Ginsberg 1985, Gosselin 1997, Bennett et al. 2007), and it is not surprising that both the lakes and temporary pools respond similarly to the climate gradient, considering that both habitats are formed by similar processes, primarily differing in size and permanence.

Environmental Characteristics

Salinity influences a variety of physical and chemical properties in aquatic systems (Hammer 1986, Moss 1994, Williams et al. 1990, Williams 1998, Nielsen et al. 2003). Differences in water quality variables between the pools was mostly influenced by or at least associated with pool salinity. The freshwater pools were similar across the Sandhills in most of the measured environmental variables except for pH. Many of the freshwater

pools in the eastern Sandhills were, on average, neutral or slightly acidic and became more alkaline westwards. Nutrient and pH levels were highly correlated with salinity and thus increased westwards as pool salinity increased. Overall, saline pools were more alkaline and contained higher nutrient levels than freshwater pools.

The unusually high nutrient levels of the saline pools have been observed in the lakes of the region (La Baugh 1986, Salm et al. 2009), and it is common for saline systems to have high phosphorus levels (Hammer 1986). The source of the phosphorus in the Sandhills waterbodies is unknown, since there is very little row-crop agriculture in the region. La Baugh (1986) found that high dissolved phosphorus concentrations in groundwater corresponded with the high phosphorus concentrations of nearby Sandhills lakes, implying that the phosphorus may be coming from the groundwater; alternatively, the groundwater could be receiving phosphorus from the lakes. It is possible that due to the thin, porous sediments of the region, phosphorus from animal waste and decaying organic matter is easily transferred through the sediments into the groundwater where it dissolves into solution, and the low calcium concentrations in the groundwater prevent the phosphorus from precipitating out of solution and forming insoluble calcium phosphate minerals (Bleed and Flowerday 1998, Hermassi et al. 2015, Oxmann and Schwendenmann 2015). Nitrogen may be high in the Sandhills due to high terrestrial N mineralization rates and N-fixation by cyanobacteria (Salm et al. 2009). Cyanobacterial mats are commonly found in saline waterbodies (Hammer 1986), and we observed them covering the bottoms of many of the saline pools that we sampled. Kapustka et al. (1988) found that high N levels in two Sandhills lakes were driven by N-fixing cyanobacteria communities within the littoral zone, and N levels were consistently higher in the

saline/alkaline lake than in the neutral lake. Thus, we speculate that cyanobacteria's affinity for saline/alkaline waters may promote higher N concentrations in the Sandhills' saline waterbodies. However, multiple lakes and wetlands ranging from freshwater to hypersaline/alkaline need to be studied in the future to test whether N-fixing cyanobacteria are driving the high N levels of the Sandhills waterbodies.

It is possible that the nutrients, salts, and carbonates may be coming from a bedrock unit that is dissolving in the ground water and later being discharged at the surface. This seems reasonable as the region was covered by an inland sea, during the late Cretaceous, and those marine strata lie beneath the Sandhills (Bleed and Flowerday 1998). However, studies on groundwater quality throughout the region suggest that Sandhills groundwater contains little carbonate and dissolved solids compared to the surrounding areas (Bleed and Flowerday 1998). Furthermore, high salinities are typically only detected in the groundwater directly beneath or around saline waterbodies, so the solutes do not appear to be travelling via the groundwater in great concentrations (Winter et al. 2001, Gosselin et al. 1994). Solute concentrations may be higher in shallower lakes and wetlands, because there is more surface area from which groundwater is discharged, and over time, high evaporation rates cause the solutes to concentrate in the water (Gosselin et al. 1994).

Many of the water quality variables fluctuated throughout the inundation period (Fig. 2; see Supplementary Data: Table 1), which was most likely driven by changes in water volume (McCulloch et al. 2008). The extremes to which many of these variables changed in the saline pools demonstrates the dynamic nature of this system. Many of the saline pools' environmental variables increased with water loss or decreased with water

gain; however, the high salinity pools were often the most variable. For example, Site 35 was our most saline pool and experienced wide fluctuations, fluctuating between 17.4 mS/cm and 37.9mS/cm; whereas, Site 26, one of the lowest saline pools, only fluctuated between 5.53 and 6.14 mS/cm. The degree to which these pools fluctuate may be an effect of water depth, and while we did not measure the depths of the pools, the freshwater pools were noticeably deeper than the saline pools, and freshwater pool water chemistry fluctuated little throughout the inundation period. Gosselin et al. (1994) found that deeper Sandhills lakes had lower solute concentrations and exhibited much less seasonal variation in solute concentrations than the shallow lakes, suggesting that shallower waterbodies in this region are more likely to have higher solute concentrations and fluctuate more with changes in water volume. Thus, while the lakes and pools are quite different in size and permanence, they do share some fundamental environmental characteristics.

Community-environment relationships

Salinity was the primary environmental gradient driving branchiopod community structure and biodiversity, and its influence on community composition appeared to increase westwards as mean salinity increased. Salinity is a strong environmental filter in inland aquatic ecosystems (Green et al. 2005, Waterkeyn et al. 2009, Viayeh and Spoljar 2012, Atashbar et al. 2014, Tavsanoglu et al. 2015, Castillo-Escriva et al. 2016); however, it is difficult to discern whether salinity is directly or indirectly structuring these aquatic communities, because salinity can influence a variety of biotic and abiotic factors (Galat and Robinson 1983; Williams 1998; Sarma et al. 2002; Santangelo et al. 2008).

For example, aquatic vegetation was less abundant and often absent in the high-salinity pools, and it is an important resource for many branchiopods as it creates heterogeneity within the waterbody, providing refuge from predators and food resources for littoral species (Boven et al. 2008; Nhiwatiwa et al. 2011; Choi et al. 2014). Therefore, it would not be surprising to find that the lack of aquatic vegetation was having some influence on branchiopod species occurrences, particularly that of halotolerant littoral cladoceran species such as *Chydorus sphaericus*, *Alona setulosa*, *S. vetulus*, *S. mucronata*, and *P. denticulatus*. To test the direct effect of salinity on species occurrences, lifetable experiments should be conducted for each species along the salinity gradient. While such studies have been conducted for many of the halotolerant cladoceran species that we collected like *C. dubia*, *D. pulex*, *M. macrocopa*, *S. vetulus*, and *S. mucronata*, (Sarma et al. 2006; Gökçe and Turhan 2014), branchiopods are often locally adapted to geographically-unique ion concentrations, so salinity tolerances will vary depending on the population and the ion-content of the saltwater (Bowen et al. 1985; Martinez-Jeronimo and Espinosa-Chavez 2005; Rogers 2014). If the species' salinity ranges exceed those observed in nature, then their occurrences are probably constrained by other factors such as dispersal, biological interactions, and other environmental factors.

Species Diversity

Alpha diversity decreased westwards in both the freshwater and saline pools. The westward decrease in saline pool biodiversity was expected, because the mean salinity increased westwards making the pools less hospitable for most species; however, the decline in freshwater biodiversity was unexpected. The western freshwater pool with the

highest alpha diversity contained fewer species than the eastern freshwater pool with the lowest alpha diversity, and the eastern freshwater gamma diversity contained twice as many species as the western freshwater gamma diversity. The freshwater pools differed little environmentally between the three study areas except for pH, which increased westwards. However, the pH levels of the western freshwater pools were still within the ranges observed in the central and eastern freshwater pools. We suspect that a combination of local and regional processes (i.e., metacommunity dynamics) (Leibold et al. 2004) may be driving these biodiversity patterns.

We hypothesize that the westward decline in biodiversity is associated with the westward increase in saline pool abundance and the associated decrease in freshwater pool abundance. The freshwater pools support a higher biodiversity than saline pools, so a change in freshwater pool abundance across the landscape would have large implications for local and regional biodiversity, particularly since temporary pools are strongly influenced by dispersal limitation (Heino et al. 2015). As the freshwater pools decrease in abundance across the landscape from east to west, the probability that a freshwater-dependent species entering the regional species pool will find a freshwater pool to colonize will decrease. From an island biogeographical perspective, isolated pools will experience lower immigration rates and in turn have a lower biodiversity than pools that are close together (MacArthur and Wilson 1963). This trend may be further exacerbated by the passive dispersal of branchiopod eggs (Brendonck and De Meester 2003). Since branchiopods rely upon wind and other animals as dispersal vectors, they are less likely to be dispersed to suitable habitats, especially if those suitable habitats are uncommon like the freshwater temporary pools in the western Sandhills (Cohen and

Shurin 2003). Future research should assess the influence that this environmental heterogeneity gradient has on actively dispersing organisms and the short-lived communities they form in the temporary pools. Since active dispersers can actively colonize suitable habitats (McPeek 1998), they may be less affected by the westward decrease in freshwater wetlands. Alternatively, active dispersers may be more constrained by the water quality of the surrounding landscape particularly that of permanent lakes, which serve as source habitats for many actively dispersing species, and the proximity of their source habitats to their sink habitats (McCauley et al. 2007; Heino et al. 2015).

Conclusion

Very little was known about the temporary pools of the Nebraska Sandhills, particularly how they differed along the east-west climate gradient that overlays the Sandhills region. We found that the temporary pools differed environmentally and ecologically across the longitudinal climate gradient, and that most of these differences were associated with salinity. Both freshwater and saline temporary pool alpha and gamma diversity declined westwards as saline pools became more frequent and salinity increased. The westward decline in saline pool biodiversity was likely driven by increases in pool salinity, as few species can tolerate those conditions. We speculate that the westward decline in freshwater pool biodiversity may be influenced by the regional decline in freshwater pools. However, since this was an exploratory study, additional surveys with larger samples sizes should be conducted to better understand these patterns. In conclusion, we find that the transition from a freshwater-dominated landscape in the eastern Sandhills to

a salt water-dominated landscape in the western Sandhills makes this system ideal for studying ecological processes at local and regional scales.

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Figure Legends

Figure 1. Map of the Nebraska Sandhills (gray), Nebraska, U.S.A. The three squares represent the sampling areas for each region: eastern, central, and western.

Figure 2. Trends in freshwater (filled circles and solid line; < 3.0mS/cm) and saline (empty circles and dotted line; ≥ 3.0mS/cm) pool environmental variables across the Nebraska Sandhills, using linear regression analyses. The x-axis represents longitudinal coordinates in which negative coordinates increase westwards. Points are seasonal means with standard error bars (n=3-4 dates per pool).

Figure 3. Partial forward selection CCA for the A) central and B) western study areas with sampling period used as a covariate. A) Eigenvalue = 0.64; explained variation = 32.6%; contribution to explained variation: conductivity = 24.9%, surface area = 7.6%. B) Eigenvalue = 0.81; explained variation = 41.9%; contribution to explained variation: conductivity = 35.6%, surface area = 6.3%. Complete species names for the species codes are provided in Supplementary Table 3.

Figure 4. Individual species' occurrences along the salinity gradient. Species are arranged along the x-axis in increasing order of occurrences. The number of occurrences for each species are listed along the x-axis at the top of the plot. Additional taxonomic information for the species is provided in Supplementary Table 3.

Figure 5. Alpha (α), beta (β), and gamma (γ) diversities of the combined (freshwater + saline), freshwater, and saline pools across the three study areas. Combined: eastern n=9, central n=11, western n=10. Freshwater: eastern n=9, central n=7, western, n=3. Saline:

eastern n=0, central n=4, western n=7. Alpha diversity was compared using One Way ANOVA's, the results of the pairwise interactions are indicated by the A-C lettering, and error bars are standard error.

Table 1. Salinity-biodiversity and surface area-biodiversity regressions of the combined freshwater and saline pool datasets, freshwater-only datasets, and saline-only datasets across the eastern, central, and western study areas. Biodiversity is measured as α-diversity. Significant P-values ($p \leq 0.05$) of the slope are in bold. NA = not applicable; N = number of pools; FW = freshwater pools; SA = saline pools.

Figure 1

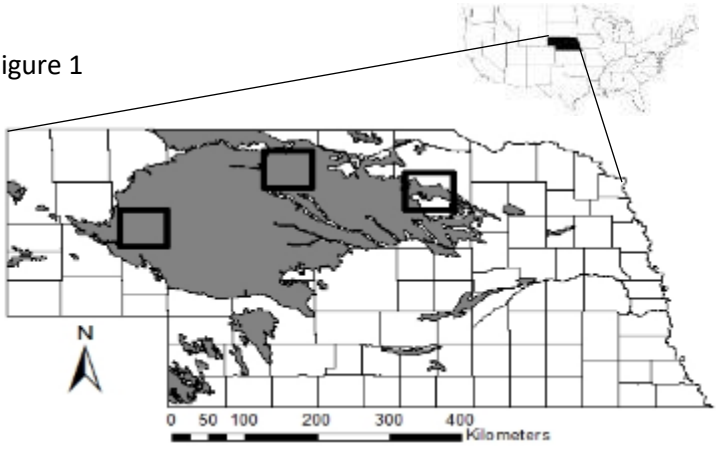

Figure 2

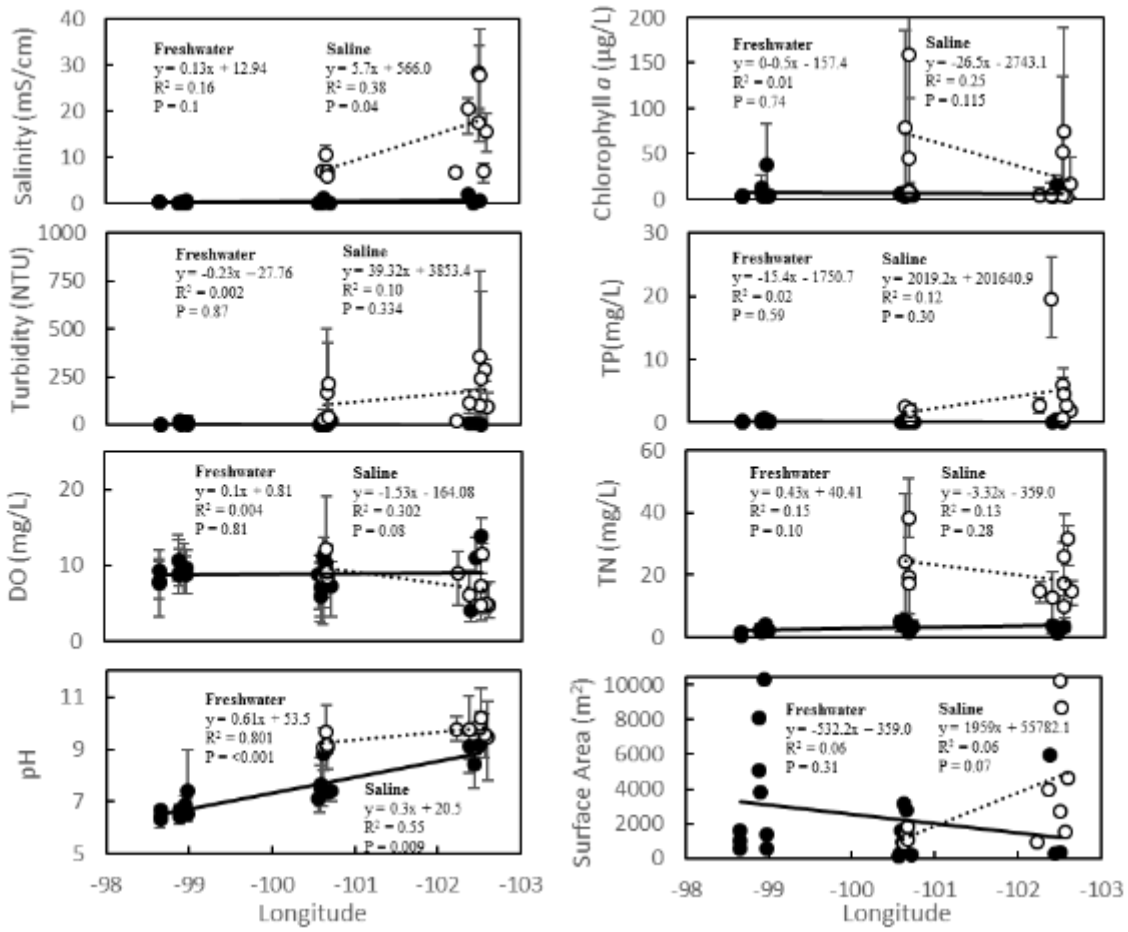
Salinity (mS/cm)
Freshwater
y = 0.13x + 12.94
R² = 0.16
P = 0.1
Saline
y = 5.7x + 566.0
R² = 0.38
P = 0.04
Turbidity (NTU)
Freshwater
y = -0.23x – 27.76
R² = 0.002
P = 0.87
Saline
y = 39.32x + 3853.4
R² = 0.10
P = 0.334
DO (mg/L)
Freshwater
y = 0.1x + 0.81
R² = 0.004
P = 0.81
Saline
y = -1.53x - 164.08
R² = 0.302
P = 0.08
pH
Freshwater
y = 0.61x + 53.5
R² = 0.801
P = <0.001
Saline
y = 0.3x + 20.5
R² = 0.55
P = 0.009
Longitude
Chlorophyll a (µg/L)
Freshwater
y = 0-0.5x - 157.4
R² = 0.01
P = 0.74
Saline
y = -26.5x - 2743.1
R² = 0.25
P = 0.115
TP(mg/L)
Freshwater
y = -15.4x - 1750.7
R² = 0.02
P = 0.59
Saline
y = 2019.2x + 201640.9
R² = 0.12
P = 0.30
TN (mg/L)
Freshwater
y = 0.43x + 40.41
R² = 0.15
P = 0.10
Saline
y = -3.32x - 359.0
R² = 0.13
P = 0.28
Surface Area (m²)
Freshwater
y = -532.2x – 359.0
R² = 0.06
P = 0.31
Saline
y = 1959x + 55782.1
R² = 0.06
P = 0.07
Longitude

Figure 3

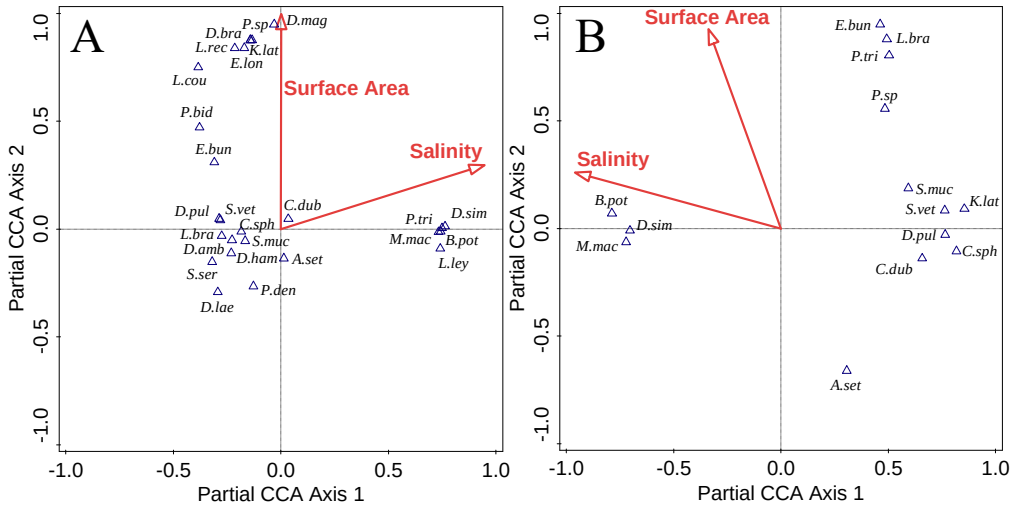
A
Surface Area
Salinity
D.mag
P.sp
D.bra
L.rec
K.lat
E.lon
L.cou
P.bid
E.bun
D.pul
S.vet
C.dub
C.sph
L.bra
S.muc
D.amb
D.ham
A.set
S.ser
P.den
D.lae
P.tri
D.sim
M.mac
B.pot
L.ley
B
Surface Area
Salinity
E.bun
L.bra
P.tri
P.sp
S.muc
K.lat
S.vet
B.pot
D.sim
M.mac
D.pul
C.sph
C.dub
A.set
Partial CCA Axis 1
Partial CCA Axis 2
-1.0
-0.5
0.0
0.5
1.0

Figure 4

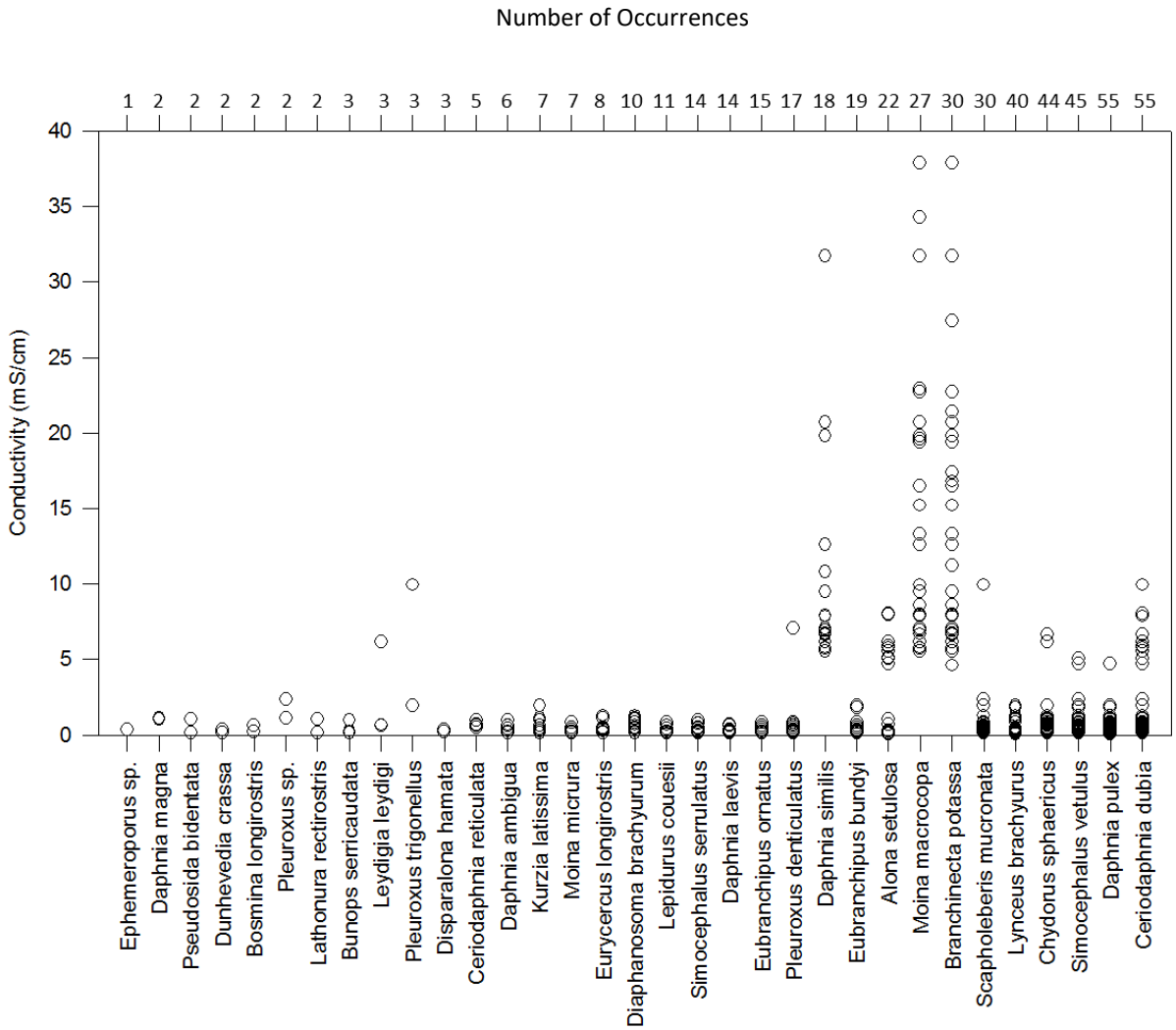
Number of Occurrences
1 2 2 2 2 2 2 3 3 3 3 5 6 7 7 8 10 11 14 14 15 17 18 19 22 27 30 30 40 44 45 55 55
Conductivity (mS/cm)
0 5 10 15 20 25 30 35 40
Ephemeroporus sp.
Daphnia magna
Pseudosida bidentata
Dunhevedia crassa
Bosmina longirostris
Pleuroxus sp.
Lathonura rectirostris
Bunops serricaudata
Leydigia leydigi
Pleuroxus trigonellus
Disparalona hamata
Ceriodaphnia reticulata
Daphnia ambigua
Kurzia latissima
Moina micrura
Eurycercus longirostris
Diaphanosoma brachyurum
Lepidurus couesii
Simocephalus serrulatus
Daphnia laevis
Eubranchipus ornatus
Pleuroxus denticulatus
Daphnia similis
Eubranchipus bundyi
Alona setulosa
Moina macrocopa
Branchinecta potassa
Scapholeberis mucronata
Lynceus brachyurus
Chydorus sphaericus
Simocephalus vetulus
Daphnia pulex
Ceriodaphnia dubia

Figure 5

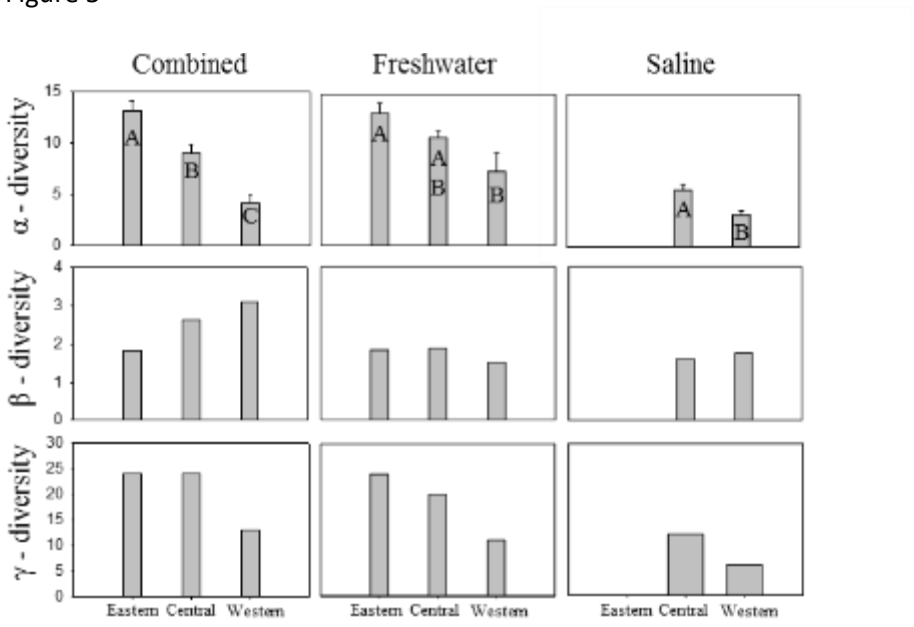
Combined
Freshwater
Saline
α - diversity
β - diversity
γ - diversity
A
B
C
A
A
B
B
A
B
Eastern Central Western
Eastern Central Western
Eastern Central Western

Table 1

Dataset	Model	N	DF	F	R^2	P
All study areas	α-diversity = 8.943 – 1.833 Salinity(mS/cm)	30	29	43.3	0.593	**<0.001**
	α-diversity = 8.403 + $9x10^{-5}$ Surface Area(m^2)	30	29	0.105	0	0.75
Eastern[1]	α-diversity = 9.530 – 2.818 Salinity(mS/cm)	9	8	3.184	0.214	0.12
	α-diversity = 11.262 + $5x10^{-4}$ Surface Area(m^2)	9	8	3.863	0.264	0.09
Central	α-diversity = 8.911 – 0.937 Salinity(mS/cm)	11	10	5.753	0.322	**0.04**
	α-diversity = 7.596 + 0.001 Surface Area(m^2)	11	10	1.842	0.08	0.208
Western	α-diversity = 6.132 – 1.069 Salinity(mS/cm)	10	9	5.412	0.329	**0.048**
	α-diversity = 4.684 – $1x10^{-4}$ Surface Area(m^2)	10	9	0.194	0	0.671
All study areas	FW α-diversity = 9.489 + $7x10^{-4}$ Surface Area(m^2)	19	18	11.516	0.369	**0.003**
Eastern	FW α-diversity = 11.262 + $5x10^{-4}$ Surface Area(m^2)	9	8	3.863	0.264	0.09
Central	FW α-diversity = 9.155 + 0.001 Surface Area(m^2)	7	6	8.175	0.545	**0.035**
Western	FW α-diversity = 5.171 + 0.001 Surface Area(m^2)	3	2	35.712	0.946	0.106
All study areas	SA α-diversity = 5.397 - $4x10^{-4}$ Surface Area(m^2)	11	10	5.298	0.301	**0.047**
Eastern[1]	NA	NA	NA	NA	NA	NA
Central	SA α-diversity = 6.227 + $2x10^{-5}$ Surface Area(m^2)	4	3	$7x10^{-5}$	0	0.994
Western	SA α-diversity = 3.519 - $1x10^{-5}$ Surface Area(m^2)	7	6	1.415	0.065	0.288

[1]All of the eastern pools were freshwater, so the regression results for surface area are the same before and after the pools were segregated by type.

CHAPTER II

INFLUENCE OF A LARGE-SCALE HETEROGENEITY GRADIENT ON CRUSTACEAN EGG BANK METACOMMUNITY STRUCTURE AND BIODIVERSITY IN TEMPORARY WETLANDS

Abstract

Dormant propagule banks play an important role in structuring communities at local and regional scales. The Nebraska Sandhills, Nebraska, supports thousands of lentic waterbodies that range from freshwater to hypersaline. These waterbodies make up a large-scale longitudinal gradient in which the abundance and salinity levels of habitats increase from east to west. Few metacommunity studies have tested the effects of large-scale heterogeneity gradients on biodiversity and metacommunity structure. Here, I used the longitudinal gradient that overlays the Nebraska Sandhills to determine how environmental heterogeneity influences the metacommunity structure and biodiversity of dormant crustacean egg banks from freshwater and saline temporary wetlands. I sampled the egg banks of 54 wetlands from three metacommunities across the Nebraska Sandhills (eastern, central, and western), mirroring and comparing the results to a previous study

that surveyed emergent crustacean communities in 2013. I found that the alpha richness of freshwater wetlands and metacommunity gamma richness declined westwards as the abundance of freshwater habitats decreased. The egg bank metacommunities from the central and western metacommunities were structured by the salinity gradient, whereas, the eastern metacommunity did not exhibit any clear patterns associated with an environmental gradient. Propagule banks may not be able to buffer local communities from environmental change as previously assumed, because they appear to be susceptible to the indirect effects of habitat loss within the metacommunity. The temporary wetlands from the Nebraska Sandhills serve as a model for the adverse effects that environmental change can have on propagule banks at local and regional scales. Future studies should incorporate other types of dormant propagule banks with metacommunity ecology to identify how different systems respond to local and regional heterogeneity gradients.

Introduction

Dormant propagule banks serve as archives of biodiversity for many terrestrial and aquatic ecosystems. Dormancy is broken by species-specific environmental cues that are associated with the species' optimal survival conditions (e.g. Cohen's Law, Cohen 1966). However, as a bet-hedging strategy, only a subset of propagules from the propagule bank will break dormancy when exposed to the appropriate environmental cues (Brendonck and De Meester 2003). Therefore, the subset of propagules that did not break dormancy will accumulate in the sediment over many years, forming a species-rich propagule bank (Hairston 1996). This strategy is particularly effective for species that

utilize temporally heterogeneous habitats, because the entire population is not lost from a rapid change in environmental conditions.

Propagule banks often consist of many species with different environmental preferences that may emerge at different times of the year or under specific environmental conditions that occur yearly or sporadically once every few years. Therefore, propagule banks often yield more species than the emergent community at any given moment (Brendonck and De Meester 2003). While these dormant propagules are used to persist through adverse conditions in the resident habitat patch, they are also essential for dispersing to new habitat patches across inhospitable landscapes (Brendonck and De Meester 2003; Wisknoski et al. 2018). Depending on the dispersal vector and sediment disturbance, propagules can be dispersed years after they were first formed (Brendonck et al 2017), therefore, the legacy of a local patch can have regional implications. Despite the importance of dormant propagule banks in structuring terrestrial and aquatic ecosystems at local and regional scales, they have never been studied from a metacommunity perspective (Wisknoski et al. 2018).

Branchiopod crustaceans occur in nearly all types of aquatic habitat (Thorp and Covich 2010) and are model organisms for studying the ecology of dormant propagule banks as they rely on resting eggs for both persisting in temporary or seasonally-dynamic aquatic habitats and dispersing to new habitats (Brendonck and De Meester 2003; Brendonck et al. 2017). Branchiopods differ widely in size, morphology, environmental tolerance, life history, functional traits, and egg morphology (Brendonck and De Meester 2003; Brendonck et al. 2008). Therefore, branchiopod egg banks are a valuable tool for studying the hidden biodiversity and past environmental conditions of aquatic

ecosystems. While branchiopods are frequently used to study the metacommunity dynamics of emergent communities, less is known about how their egg banks influence species composition at local and regional scales.

The Nebraska Sandhills, Nebraska, USA is one of the largest grass-stabilized dune systems in the western hemisphere and is overlain by a longitudinal climate gradient that is typical of the Great Plains region (Bleed and Flowerday 1990). The topography of the Nebraska Sandhills allows for the formation of groundwater-fed interdunal lakes and wetlands that range from freshwater to hypersaline (Bennett et al. 2007; Mausbach and Dzialowski 2017). Due to the longitudinal climate gradient, the abundance and salinity levels of saline waterbodies increases westwards as the climate becomes more arid (Ginsberg 1985; La Baugh 1986; Gosselin et al. 1994; Gosselin 1997; Mausbach and Dzialowski 2017), producing a landscape-level gradient in contrast-based environmental heterogeneity (Mausbach and Dzialowski 2017). Salinity plays a strong role in structuring aquatic communities, and inland saline waters are often species poor as species diversity decreases with increases in salinity (Hammer 1986; Moss 1994; Williams 1998; Mausbach and Dzialowski 2017). Therefore, salinity is an ideal variable from which to study the influence of environmental gradients on metacommunity structure and biodiversity, because it affects a variety of chemical, physical, and biological processes in aquatic communities, producing distinct contrasts between habitats at different salinity levels (Williams et al 1990; Williams 1998, Mausbach and Dzialowski 2017).

Salinity levels vary temporally depending on precipitation and evaporation rates, and saline waterbodies can shift between freshwater and saline conditions, supporting

vastly different communities (personal observation, Green et al 2005). Therefore, temporally heterogeneous saline wetlands are likely to support species-rich egg banks that contain freshwater, halo-tolerant (freshwater species that tolerate low salinities), and halophilic (salt-loving) species. Mausbach and Dzialowski (2017) surveyed temporary wetlands across the Nebraska Sandhills and found that the biodiversity of the emergent branchiopod communities from saline wetlands decreased with increases in wetland salinity and that the saline wetlands were species-poor compared to their neighboring freshwater wetlands. Furthermore, freshwater wetland species richness declined westward as freshwater wetlands became less common across the landscape, suggesting that freshwater communities are dispersal-limited (Mausbach and Dzialowski 2017). The Nebraska Sandhills vary widely in annual precipitation (Ginsberg 1985), therefore, a survey of the emergent community from a single year may not be indicative of true biodiversity trends across the region. My objective for this study was to conduct a survey for branchiopod egg banks from freshwater and saline wetlands across the Nebraska Sandhills to determine if the egg banks exhibit similar biodiversity patterns as the emergent communities from Mausbach and Dzialowski (2017). I also aimed to determine whether both the egg bank and emergent community datasets produced similar metacommunity structure patterns by using the Elements of Metacommunity Structure (EMS) analysis (Leibold and Mikkelson 2002; Table 1).

I hypothesized that freshwater egg banks would exhibit a westward decline in alpha richness like the emergent community, because the freshwater wetland communities are likely dispersal limited due to the low abundance/ high isolation of freshwater wetlands across the landscape in the western Sandhills. I hypothesized that the

egg bank richness of low-salinity wetlands would be higher than that of neighboring freshwater wetlands and of the saline emergent communities, because they will support species from both freshwater and saline communities as they can fluctuate between freshwater and saline environments annually. Therefore, the egg bank alpha richness of low-salinity wetlands will not follow the negative relationship with salinity observed with the emergent communities in Mausbach and Dzialowski (2017). However, I expect that high-salinity wetlands will still exhibit a decrease in alpha richness of egg banks, because high-salinity wetlands are less likely to shift to freshwater conditions under high precipitation, therefore, they will only support halo-tolerant and halophilic species. I hypothesized that the emergent metacommunities would shift from a random structure (Table 1) in the eastern Sandhills where there is no strong environmental gradient to more defined structures in the central and western Sandhills (Table 1) in which species-turnover will occur along the salinity gradient, producing transitional and/or discrete communities. I also hypothesized that the egg bank metacommunity structures would follow a similar trend as the emergent metacommunities, however, the central and western metacommunities would display gradual species turnover along the salinity gradient rather than discrete species groupings, because the egg banks will support species across the salinity gradient (Table 1).

Methods

In August 2014, I sampled the egg banks of 54 temporary wetlands across three study areas in the Nebraska Sandhills (eastern: n = 18, central: n = 18, western: n = 18), each study area will hereon be referred to as individual metacommunities. While the

borders of natural metacommunities are not always discrete, particularly in a continuous landscape covered in habitat patches like the Nebraska Sandhills, branchiopod dispersal is severely limited at distances greater than 20km from the source population (Havel and Shurin 2004). The three metacommunities were separated by at least 150km. While there are wetlands distributed between these metacommunities, dispersal between the metacommunities is unlikely to be occurring at ecologically relevant timescales. Sediment samples were collected from three points in each wetland: the wetland shore, the wetland center, and halfway between the two. The sediment samples consisted of the top 1-2 cm of sediment from a 10x10cm square at each point. Unlike most lakes, wetland sediments are frequently mixed by animal traffic and wave action, therefore, the egg banks are not chronologically layered in the sediment, but rather mixed with individuals from different years and environmental conditions. The samples were stored in freezer bags and kept in the dark at 4°C. While in storage, the bags were left open so that the samples could continue to dry. I measured the relative salinity of each site by placing 2L of homogenized dried sediment into 37.9 L aquaria. I stirred the solutions daily for four days to allow all the salts to dissolve and then measured the salinity of each solution.

I extracted eggs from 500mL of homogenized sediment following a modified protocol from Marcus (1990). I mixed the 500mL of sediment with deionized water in a plastic dish pan to separate the eggs from the sediment. Since many of the sediment samples consisted of sand, the sand would settle to the bottom and the eggs and vegetation debris would often float or take longer to settle out of the water column. The suspended eggs and debris were poured through a series of three sieves: 2mm, 250μm, and 45μm. The material collected in the 2mm sieve was discarded, while the material in

the 250μm was transferred to 50mL centrifuge tubes that were filled with sugar water solution (Marcus 1990). The samples in the sugar solution were spun at 4,000rpm for 2 minutes. After the solution was centrifuged, the floating debris and eggs were poured into a 63μm sieve, rinsed, transported into a petri dish, and the sediment at the bottom of the tubes were discarded. Due to the small amount of material that was collected in the 45μm sieve, the material was not centrifuged, but instead transferred directly to the petri dish. The sediment in the dish pan was washed multiple times until there was visibly no more suspended eggs or debris. I removed as much water from the petri dishes as possible with a pipette and then left them to air-dry. Once dried, the petri dishes were stored in a freezer until the samples were processed. I sorted the eggs into well plates so that each egg-type had its own well and each well was photographed. Eggs were identified to genus and species when possible through hatching of the ephippia, collecting ephippia from adults cultured from wild populations, and from descriptions in the literature (Goulden 1968; Vandekerkhove et al 2004; Mergeay et al 2005). *Ceriodaphnia*, *Simocephalus,* and *Eubranchipus* were the only genera that I could not identify to the species-level, and there were six ephippia for which I was unable to identify to genus or family, though I suspect two of them belong to the family *Macrothrixidae* and the other four belong to the family *Chydoriidae*.

The emergent community dataset from Mausbach and Dzialowski (2017) consists of species occurrences from temporary wetlands sampled from the same three study areas indicated in Figure 1. Mausbach and Dzialowski (2017) collected emergent branchiopods from 30 temporary wetlands with a 250 micrometer-mesh dip-net with 3-minute sweeps throughout the water column and available microhabitats once a month for 3-4 months,

depending on wetland inundation length. The branchiopods were identified to species and recorded as present or absent. Many of the same wetlands were sampled for both emergent (Summer 2013) and dormant (Summer 2014) branchiopod communities.

Analytical Methods

I did a regression of how log-transformed soil conductivity varied with longitude between the freshwater and saline wetlands. I classified the wetlands as freshwater or saline *a priori* in the field based on the visible characteristics of the wetlands that included: the presence/absence of evaporite salts and aquatic vegetation (Mausbach and Dzialowski, 2017). Low-salinity wetlands can fluctuate between freshwater and saline conditions depending on the precipitation regime of a given year, so their egg banks are likely to support a higher biodiversity than high-salinity wetlands, which remain saline even under high precipitation (personal observation). Thus, it was important to further classify the saline wetlands as low-salinity (< 2mS/cm) and high-salinity (> 2mS/cm) groups when analyzing differences in species richness, which was based on a combination of the relative salinities of the sediment samples and the presence of *Artemia franciscana,* which is associated with hypersaline environments (Bowen et al. 1988). I used a Kruskal-Wallis One-way Analysis of Variance on Ranks to compare the egg bank alpha richness of the low-salinity, high-salinity, and freshwater wetlands across the metacommunities. I compared egg bank and emergent alpha richness within habitat types (egg bank freshwater vs emergent freshwater and egg bank low-salinity vs emergent saline) and metacommunities (eastern, central, and western) using t-tests or Mann-Whitney Rank Sum tests for nonparametric data to determine whether egg banks support

a higher diversity than their ecologically equivalent emergent communities. I only compared the saline communities from the emergent dataset to the low-salinity egg bank communities, because the same saline wetlands from Mausbach and Dzialowski (2017) were used for the egg bank survey and were classified as low-salinity wetlands based on the relative salinities of the sediments. The high-salinity wetlands from the egg bank survey had not been sampled previously, therefore, I do not have emergent communities with which to compare them.

I used the elements of metacommunity structure (EMS) analysis (Leibold and Mikkelson, 2002) with the R package: *metacom* (Dallas, 2014) in R 3.0.3 to identify the metacommunity structure of each study area. I performed this analysis on both the emergent community data from Mausbach and Dzialowski (2017) and the egg bank data to determine if the two community measures produced similar metacommunity structures. The EMS framework classifies metacommunities into one of six structures (checkerboard, random, nested, Gleasonian, evenly-spaced, and Clementsian; Table 1) by applying three statistics (coherence, turnover, and boundary clumping) to species-by-site matrices that have been ordered by reciprocal averaging. I followed the guidelines from Presley et al. (2010) in which nestedness, evenly-spaced, Gleasonian, and Clementsian structures may be classified as quasi-structures based on the interaction of the turnover and boundary clumping statistics (Table 1).

Results

Of the 54 sampled wetlands, 34 were freshwater (eastern = 18, central = 11, and western = 5), 15 were low-salinity (central = 7 and western = 8), and 5 were high-salinity

(western = 5). Conductivity significantly increased westwards in both freshwater and saline wetlands (Fig. 2; Freshwater, $p = 0.001$, $F_{1,33} = 12.121$; Saline, $p = 0.002$, $F_{1,17} = 13.975$) with the conductivity appearing to be more variable amongst saline wetlands in the western Sandhills than in the central Sandhills (Fig. 2).

Thirty-three egg/ephippia morphotypes were identified (see Appendix 1) and used for estimating alpha and gamma richness. I was able to identify 23 of the morphotypes to species, 2 to genus, and the remaining 8 to family. Due to indiscernible differences between the ephippia and egg morphologies of some genera (e.g. *Ceriodaphnia*, *Simocephalus*, and *Eubranchipus*) my analyses of species richness in the egg bank likely underestimated the true richness of the sites, because these genera were treated as if they only contained one species each, even though multiple species often co-occur in Sandhills wetlands (Mausbach and Dzialowski 2017). Furthermore, the tadpole shrimp, *Lepidurus couesii*, and the clam shrimp, *Lynceus brachyurus*, were frequently found in freshwater wetlands by Mausbach and Dzialowski (2017); however, their eggs were not detected in the sediment samples, further suggesting an underestimate of egg bank diversity.

The alpha richness of the egg bank and emergent communities were influenced by multiple factors. The alpha richness of the egg banks from freshwater wetlands significantly declined westward (Fig. 3), and there were no significant differences between the emergent and egg bank alpha richness of the freshwater wetlands within the metacommunities (Fig. 4; T-test, $p > 0.05$). The egg banks from low-salinity wetlands were not significantly different between study areas (Fig. 3); however, the egg banks exhibited a higher alpha richness than the low-salinity emergent communities from

Mausbach and Dzialowski (2017) (Fig. 4; T-test, $p < 0.001$). The mean alpha richness of freshwater and low-salinity wetlands was not significantly different within the study areas; however, some low-salinity wetlands did contain more species than neighboring freshwater wetlands (Fig. 3). Gamma richness appeared to decline westward from 27 species in the eastern Sandhills to 24 and 20 species in the central and western Sandhills respectively.

The emergent and egg bank metacommunity structures responded differently to the longitudinal heterogeneity gradient (Table 2). The emergent metacommunity from the eastern Sandhills was classified as having a random metacommunity structure, implying that species occurrences are not being driven by environmental gradients or strong biological interactions. The eastern egg bank metacommunity exhibited a Clementsian structure, indicating that discrete communities are forming along an environmental gradient; however, no variables other than conductivity were recorded for these wetlands, so we are unsure as to what the environmental gradient may be and ordination analysis from Mausbach and Dzialowski (2017) indicates that there are no influential environmental gradients structuring emergent communities in the eastern Sandhills (Table 2, Fig. 5). The emergent metacommunity from the central Sandhills produced a quasi-Gleasonian structure in which species turnover occurred gradually along the salinity gradient (Table 2, Fig. 5). Gleasonian and quasi-Gleasonian structures primarily differ in that species in quasi-Gleasonian metacommunities often have wide niche breadths that can lead to positive, but non-significant turnover (Presley et al 2010). The egg bank metacommunity from the central Sandhills produced a Clementsian structure that was driven by the salinity gradient, forming 2 distinct community groups: freshwater

and saline (Table 2, Fig. 5). The egg banks from the central saline wetlands supported 9 species that were found in both freshwater and saline wetlands and 3 species that are typically associated with freshwater but were only found in saline wetlands (Fig. 5). Both the emergent and egg bank metacommunities from the western Sandhills exhibited Clementsian structures (Table 2) that corresponded with the salinity gradient; however, the egg bank metacommunity consisted of three community groups (freshwater, low-salinity, and high-salinity), whereas, the emergent community only consisted of two (freshwater and low-salinity) (Fig. 5). However, the difference in community groups between the emergent and egg bank datasets in the western Sandhills metacommunity is likely the result of Mausbach and Dzialowski (2017) not having sampled the emergent communities from high-salinity wetlands.

Discussion

Egg banks are ecological archives and reservoirs of biodiversity for inland waters. Despite their unequivocal role in structuring communities across space and time, they have not yet been studied from a metacommunity perspective (Wisknoski et al. 2018). Here, I conducted a survey of branchiopod egg banks from temporary wetlands to determine how egg bank diversity and metacommunity structure respond to the large-scale increases in environmental heterogeneity, and whether these patterns resemble those that have been observed in emergent branchiopod communities.

The emergent and egg bank metacommunities exhibited different patterns in species richness. The egg banks of the low-salinity wetlands supported more species than the emergent saline communities as was expected (Fig. 4), but they did not support more

species than their neighboring freshwater wetlands (Fig. 3). Many of the species that were found in the low-salinity egg banks were those that were either halotolerant or halophilic, so even though these low-salinity wetlands occasionally reach freshwater conditions, they may not do so often enough to support more sensitive species. The freshwater egg banks exhibited a similar westward decline in alpha richness as the emergent communities (Mausbach and Dzialowski 2017) that corresponded with an increase in environmental heterogeneity (Fig. 3).

The westward decline in freshwater wetland biodiversity is intriguing. Both the theory of island biogeography and the species-area heterogeneity tradeoff theory predict that biodiversity should decline in landscapes with fewer suitable habitat patches (i.e. freshwater wetlands) due to dispersal limitation, whether it be from limiting colonists from reaching new habitats or reducing the probability of rescue effects preventing localized extinctions (MacArthur and Wilson 1963; Allouch et al 2012; Resetarits et al 2018). However, the aquatic habitats from the western Sandhills would have originally been freshwater and became saline over centuries of excessive evaporation (Gosselin 1997). Hypothetically, the egg banks should have buffered the freshwater wetlands from local extinctions, even if those wetlands were subject to dispersal limitation from the regional species pool. Sferra et al (2017) surveyed 15 permanent Alabama ponds and found a unimodal relationship between pond age and zooplankton species richness and speculated that older ponds are species poor due to the dominance of superior competitors, so it is possible that, if given enough time, local competition could deplete the egg bank of competitively inferior species. The best approach for addressing this issue would be to collect sediment cores from freshwater and saline lakes from across the

Nebraska Sandhills to assess changes in branchiopod community composition and richness over time. Lakes and wetlands share many of the same branchiopod species, and temporary wetlands are the primary sources of branchiopod immigrants for permanent waterbodies (Brendonck et al 2017).

Few studies have documented patterns in metacommunity structure across multiple natural metacommunities (Presley and Willig 2010; Henriques-Silva et al. 2013; Heino et al. 2015a; Heino et al. 2015b), and those that were, have focused on terrestrial or permanent aquatic systems. Here, I analyzed the changes in metacommunity structure along a large-scale environmental heterogeneity gradient for both egg bank and emergent community datasets. As expected, metacommunity structure was influenced by the environmental heterogeneity gradient that overlays the Nebraska Sandhills (Bennet et al 2007; Mausbach and Dzialowski 2017). The emergent metacommunity from the eastern Sandhills exhibited a random structure (Table 2, Fig. 5), implying either 1) that species occurrences were not driven by an underlying environmental gradient, or 2) that species responded to multiple environmental gradients instead of a single environmental gradient (Presley et al. 2010). The first option seems more likely, since Mausbach and Dzialowski (2017) found that environmental variables did not explain the variation between the emergent communities. However, it is possible that the eastern metacommunity was structured by multiple gradients that I did not measure, or that the environmental gradients were too weak and my sample size too small to detect how those gradients influenced species occurrences (Heino 2005; Presley et al 2009). Random metacommunity structures are uncommon (Leibold and Mikkelson 2002; Henriques-Silva et al 2013; Heino et al 2015b) and more likely to arise in passive-dispersing

organisms like algae, bacteria, flightless invertebrates, and plants (Heino 2013; Heino et al 2015a; Heino et al 2015b). However, it is interesting that the eastern egg bank metacommunity produced a Clementsian structure (Table 2, Fig. 5), which is usually indicative of strong, local species-sorting. Many of the species from the eastern egg banks were widely distributed across many sites, and it appears that the occurrences of a few rare species may be responsible for producing the Clementsian structure (Fig. 5). Presley and Willig (2010) found that most bat metacommunities in the Caribbean that exhibit Clementsian structures are driven by dispersal limitation rather than environmental gradients.

The egg bank and emergent metacommunities from the central and western Sandhills were structured by the salinity gradient (Table 2, Fig. 5) as was expected. Both the central and western egg bank metacommunities exhibited Clementsian structures rather than Gleasonian structures, which I did not predict. The central egg bank metacommunity consisted of two community types: freshwater and saline; whereas, the western egg bank metacommunity consisted of three community types: freshwater, low-salinity, and high-salinity (Fig. 5). The lack of Gleasonian structures in the egg bank metacommunities may be due to wide niche breadths of the species within those communities. Many of the halotolerant species such as *D. pulex*, *Ceriodaphnia spp*, *Simocephalus spp*, and *Alona salina* co-occurred in the same saline wetlands (Fig. 5). Therefore, they may exhibit similar salinity tolerances so that turnover from low- to high-salinity communities is abrupt rather than gradual. Also, the grouping of some of these species into single taxa may have unrealistically broadened their niche breadths, when species within the same genus can exhibit strikingly different salinity tolerances

(Goulden 1968; Aladin 1991). Wen and Zhi-Hui (1999) found similar patterns in saline habitats in China in which biodiversity was negatively correlated with salinity, but it did not decline in a predictable manner, indicating that groups of species exhibited similar tolerances. Clementsian structures are one of the most common structures found in natural metacommunities (Henriques-Silva et al 2013; Heino 2015b), where species are sorted by a strong underlying environmental gradient, which was present in the Nebraska Sandhills. Henriques-Silva et al (2013) found that Clementsian structures were more common in lake metacommunities in which species occurrences were driven by the local environment. However, Clementsian structures have also been found in metacommunities with low levels of environmental heterogeneity (Heino et al 2015a) such as the eastern egg bank metacommunity in this study.

Species-environmental associations varied between metacommunities, highlighting that multiple processes are at work in structuring these temporary wetlands and that sampling multiple metacommunities is important when trying to understand the role of environmental gradients on local and regional species compositions. There were multiple instances between egg bank metacommunities in which species were regionally present, but not in the same habitat types across all three metacommunities. For example, *Dunehevidia crassa* was common in freshwater wetlands in the eastern Sandhills, common in saline but absent in freshwater wetlands in the central Sandhills, and common in freshwater but absent in saline wetlands in the western Sandhills (Fig 4). *Macrothrix rosea*, *Lathonura rectirostris* and *Leydigia leydigi* were found in the freshwater wetlands of the eastern Sandhills, but then became more prevalent in the saline wetlands of the central and western Sandhills (Fig. 5). Many species were consistently found in similar

environments across two or three of the study areas and can therefore be used to infer the temporal heterogeneity of Sandhills wetlands in regards to salinity. *Daphnia similis*, *Moina macrocopa*, and *Branchinecta potassa* were iconic of most saline wetlands, particularly low-salinity wetlands, whereas, *Moina hutchinsoni* and *Artemia franciscana* were representative of high-salinity wetlands (Fig. 5). *Scapholeberis mucronata*, *Daphnia laevis*, *Daphnia magna*, *Eulimnadia diversa*, *Eubranchipus spp.*, and *Bunops serricaudata* only occurred in freshwater wetlands and were detected across the three metacommunities (Fig. 5). While these species groups in conjunction with the salinity ranges observed in Mausbach and Dzialowski (2017) could be used to infer past environmental conditions in lake sediments, it is important to acknowledge that branchiopods can become locally adapted (Bowen et al 1985; Pantel et al 2015; Rogers 2015; Frisch et al 2017) and using a single population to infer the environmental tolerances of other contemporary or ancient populations has limitations. For example, Frisch et al. (2017) found that *Daphnia* phenotypes can differ greatly between two contemporary populations that were exposed to different levels of historical environmental change. Therefore, it is possible that some of the inconsistent species-environmental associations across the different Sandhills metacommunities are driven by local adaptations. Future lifetable studies should be conducted on species with different apparent salinity tolerances from both freshwater and saline populations within and between metacommunities to determine whether local adaptation is influencing community composition.

The westward decline in freshwater biodiversity in both the emergent and egg banks of these wetlands has broad implications for the biodiversity of aquatic and

terrestrial ecosystems globally. Propagule banks are important sources of colonists for many terrestrial and aquatic ecosystems, making them an intrinsic component of metacommunity ecology. However, as I found in this study, propagule banks may not be able to buffer local communities from environmental change as previously assumed, because they appear to be susceptible to the indirect effects of habitat loss within the metacommunity. The temporary wetlands from the Nebraska Sandhills serve as a model for the adverse effects that environmental change can have on propagule banks at local and regional scales. Future studies should incorporate other types of dormant propagule banks with metacommunity ecology to identify how different systems respond to local and regional heterogeneity gradients.

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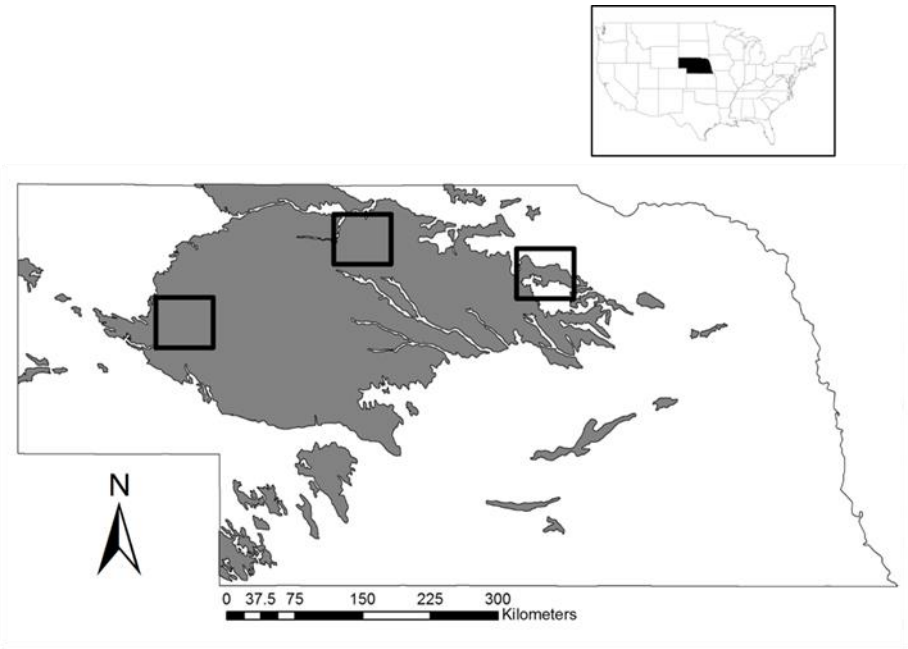

Figure 1. Map of the Nebraska Sandhills (gray), Nebraska, U.S.A. The three squares represent the sampling areas for each metacommunity: eastern, central, and western.

Table 1. Metacommunity structures with their descriptions and the statistical results required for each structure. The − and + indicate whether the results of each analysis were higher or lower than the null, S represents statistical significance ($p \leq 0.05$), and NS represents non-significance ($p > 0.05$).

Structures	Descriptions	Coherence	Turnover	Boundary Clumping
Checkerboard	Species pairs have mutually exclusive distributions	- , S	NA	NA
Random	Species distributions do not follow any particular gradient	NS	NA	NA
Nested	A pattern in species loss across communities in which species poor sites contain subsets of species from species rich sites	+ , S	- , S	NA
Clumped	Groups of species are lost between communities	+ , S	- , S	+ , S
Stochastic	Species are sporadically lost between communities	+ , S	- , S	NS
Hyperdispersed	Species are steadily lost between communities	+ , S	- , S	- , S
Evenly-Spaced	Species do not form discrete communities, but species ranges are arranged more evenly than what would be expected by chance	+ , S	+ , S	- , S
Gleasonian	Species ranges are structured idiosyncratically along an ecological gradient	+ , S	+ , S	NS
Clementsian	Discrete communities form along an ecological gradient without overlapping with other communities	+ , S	+ , S	+ , S
Quasi-Structures	Contain the same basic characteristics of the original structures, except that turnover is not significantly different from random	--	NS	--

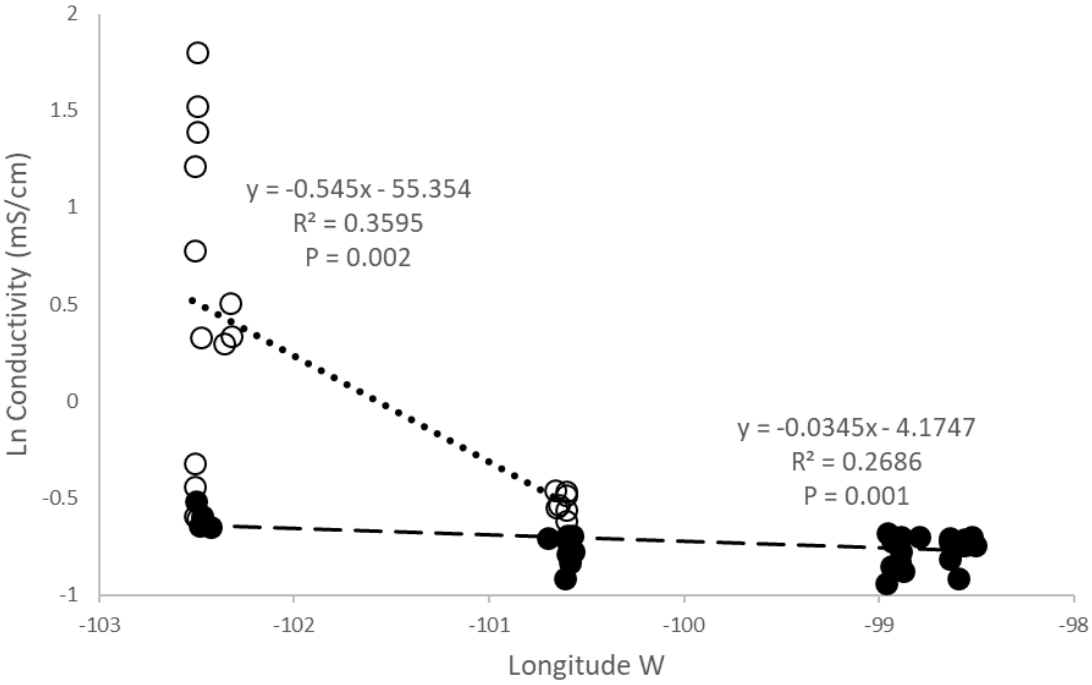

Figure 2. Linear regressions comparing the relative soil conductivities of freshwater (black circles, dashed line) and saline (empty circles, dotted line) wetlands across the longitudinal gradient. Saline and freshwater designations were assigned in the field based on visible characteristics, therefore, there is some overlap in the conductivities of some saline and freshwater sites.

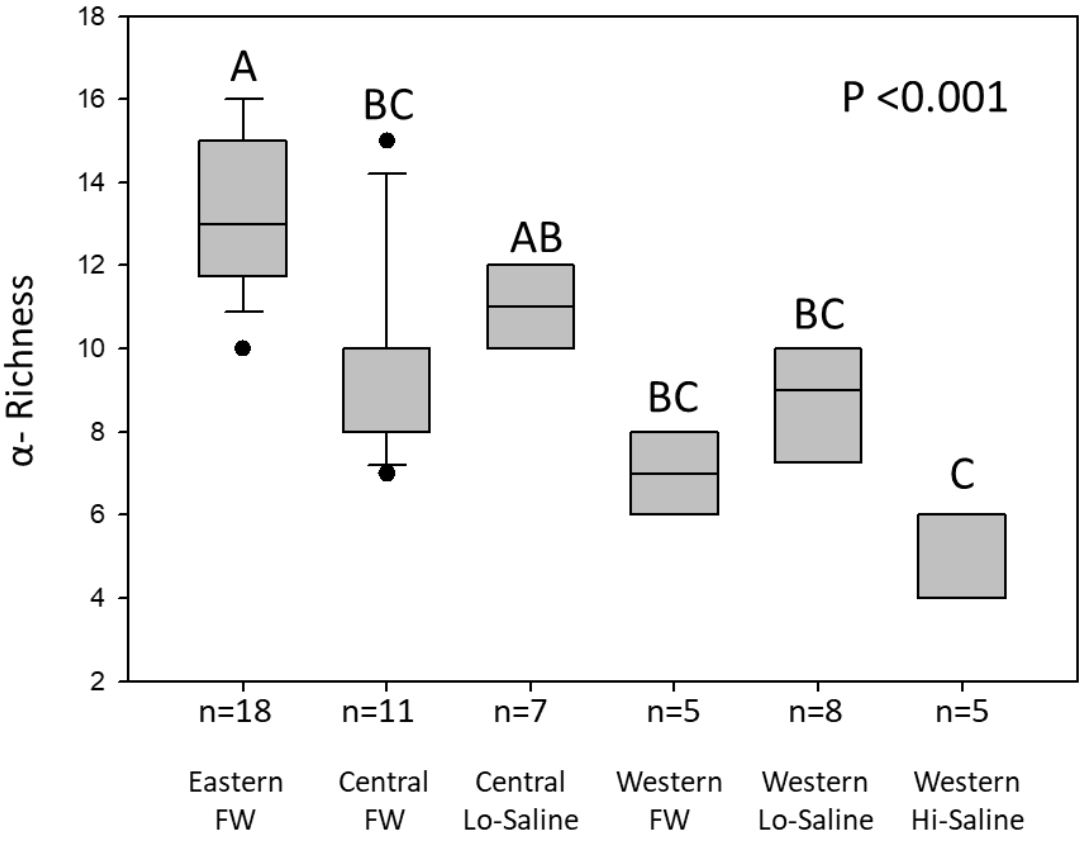

Figure 3. Alpha (α) richness of freshwater, low-salinity, and high-salinity wetland egg banks from the eastern, central, and western Nebraska Sandhills. Alpha diversity was compared using a Kruskal-Wallis One-way Analysis of Variance. The results of the pairwise interactions are indicated by the A-C lettering.

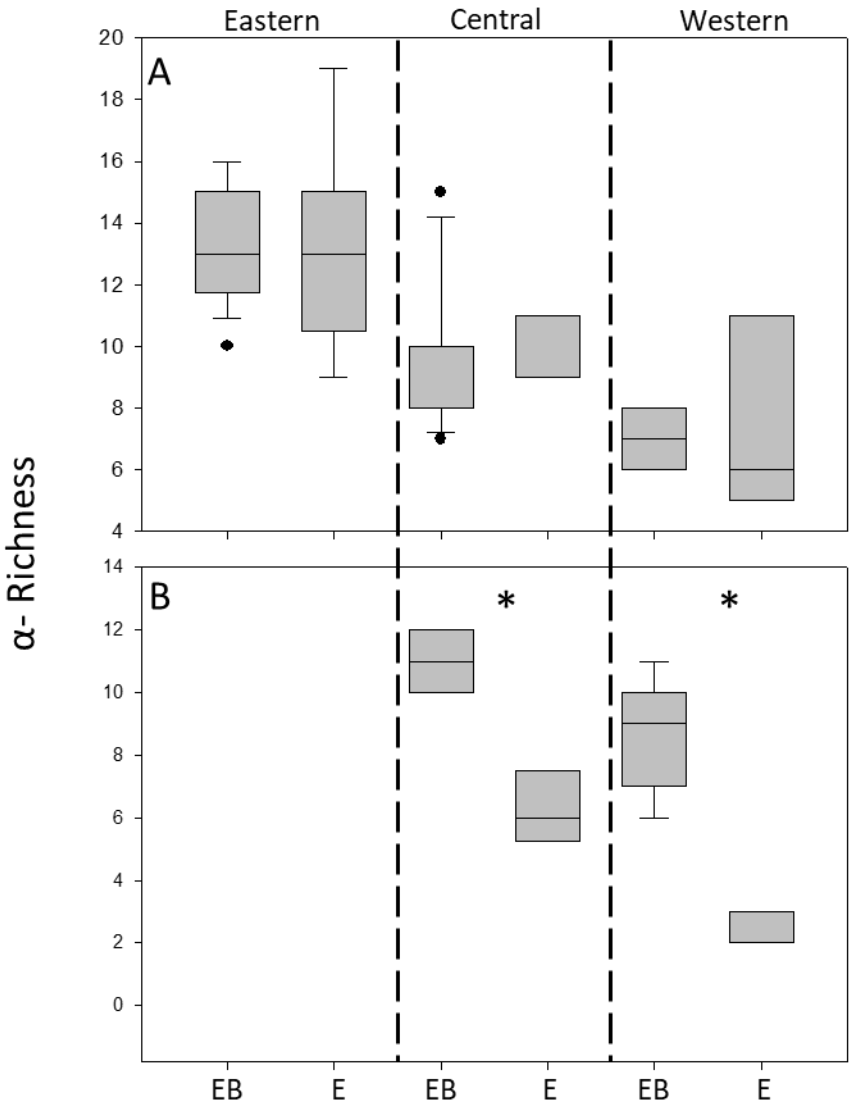

Figure 4. Egg bank (EB) and emergent (E) community α-richness in freshwater (A) and low-salinity (B) wetlands from the eastern, central, and western Nebraska Sandhill metacommunities. Egg bank and emergent alpha diversity were compared within metacommunities using t-tests or Mann-Whitney Rank Sum tests for nonparametric data. Significant differences are indicated by *.

Table 2. EMS results for each metacommunity. The − and + indicate whether the results of each analysis were higher or lower than the null, S represents statistical significance ($p = 0.05$), and NS represents non-significance ($p > 0.05$).

Region	Data Source	Metacommunity Structure	Coherence	Turnover	Boundary clumping
Eastern	Emergent	Random	−, NS	+, NS	−, S
	Egg Bank	Clementsian	+, S	+, S	+, S
Central	Emergent	Quasi-Gleasonian	+, S	+, NS	+, NS
	Egg Bank	Clementsian	+, S	+, S	+, S
Western	Emergent	Clementsian	+, S	+, S	+, S
	Egg Bank	Clementsian	+, S	+, S	+, S

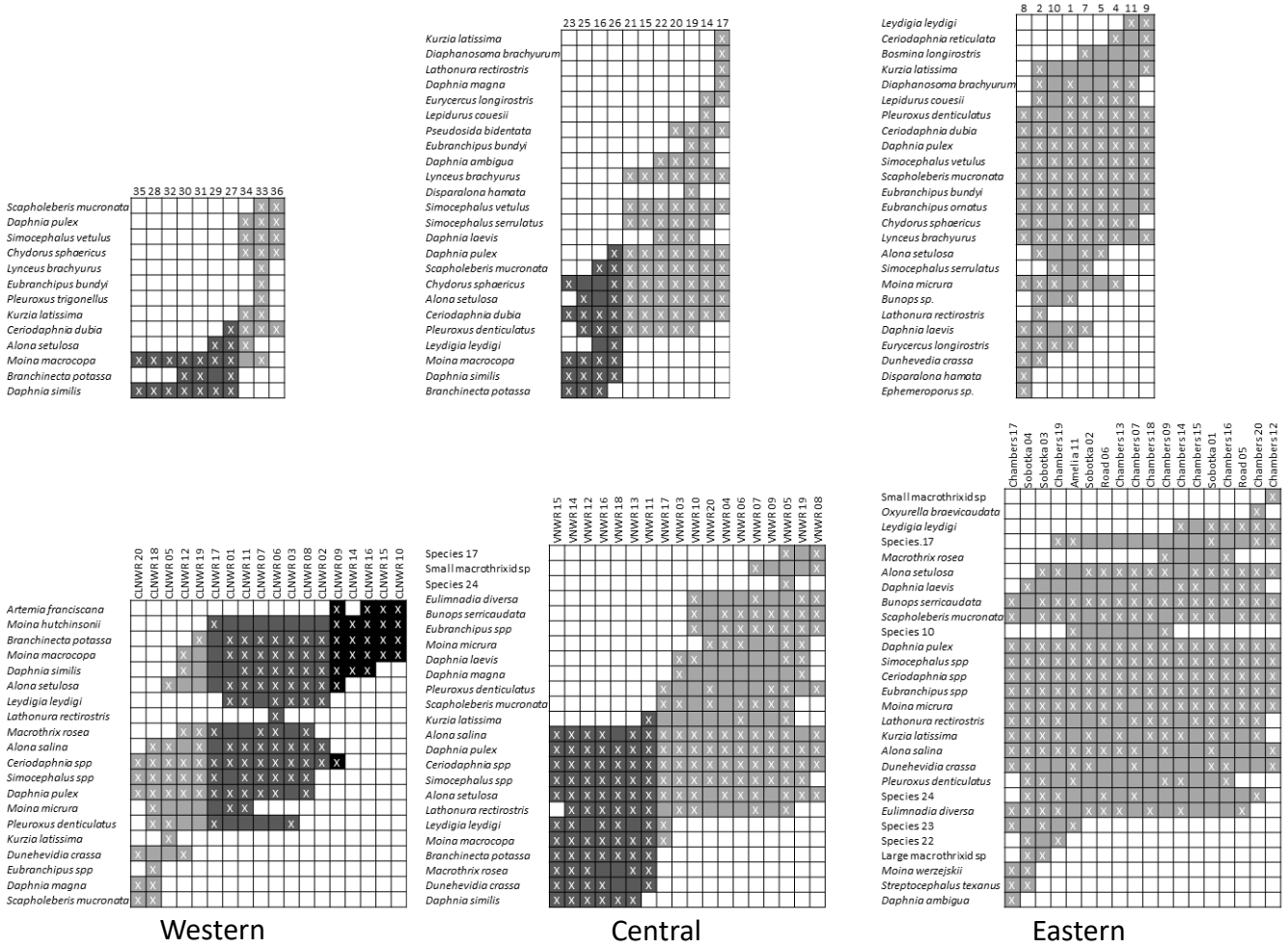

Figure 5. Species-site matrices ordered by reciprocal averaging for each of the three metacommunities. Columns are wetlands and rows are species. Top row consists of emergent data from Mausbach and Dzialowski (2017) and bottom row consists of egg bank data. Shading indicates habitat type: Light gray = freshwater, dark gray = low salinity, black = high-salinity. The white X's indicate actual species occurrences. Species absences between the occurrences were filled-in to indicate potential occurrences and niche breadth.

CHAPTER III

HABITAT IDENTITY AND SPATIAL HETEROGENEITY INTERACT TO DRIVE BIODIVERSITY IN EXPERIMENTAL METACOMMUNITIES STRUCTURED ALONG A SALINITY GRADIENT

Abstract

Environmental heterogeneity is an important component of metacommunity ecology, however, different types of heterogeneity are likely to have different impacts on metacommunity dynamics. Field surveys of freshwater and saline wetlands from the Nebraska Sandhills, Nebraska, suggest that shifts from freshwater- to saline-dominated metacommunities are causing declines in freshwater wetland alpha richness and metacommunity gamma richness. I tested how spatial heterogeneity and the environmental contrast between freshwater and saline habitat patches influences zooplankton biodiversity and species composition at local and regional scales in experimental mesocosms. I found that alpha richness of freshwater mesocosms and metacommunity gamma richness declined in saline-dominated metacommunities regardless of the environmental contrast between the freshwater and saline mesocosms. I found that environmental contrast influenced freshwater community composition in

metacommunities containing low-salinity and freshwater mesocosms by increasing the abundances of species that could tolerate low-salinity environments in freshwater mesocosms. Freshwater mesocosms had a disproportionate effect on the local and regional biodiversity in these experimental metacommunities, indicating that habitat identity may be more important than habitat diversity for maintaining biodiversity in some metacommunities.

Introduction

Environmental heterogeneity is an important component of metacommunity ecology that structures local communities and regional species pools (Leibold et al 2004; Logue et al 2011; Biswas and Wagner 2012). Heterogeneity is an ambiguous term that represents a variety of conditions or patterns, whether it be differences along an environmental gradient, habitat-patch type, abundance, or spatial orientation (Seiferling et al 2014); therefore, studies on biodiversity-heterogeneity relationships often yield conflicting results, in which heterogeneity is found to either increase (Davies et al 2009; Priego-Santander et al 2013; Bar-Massada and Wood 2014) or decrease (Forbes and Chase 2002) biodiversity. While some of these conflicting relationships can be attributed to the organisms and spatial scales (Tews et al 2004; Bar-Massada 2015), the type of environmental variables used to produce heterogeneous patterns also plays an important role. Heterogeneity is often represented by differences in resource abundance in both theoretical (Mouquet and Loreau, 2003; Mouqet et al, 2006) and empirical (Forbes and Chase, 2002; Davies et al, 2009; Mathiessen, et al 2010, Foster et al, 2011; Resitarits et al 2018) studies. While there is support for resource-based species-sorting (Interlandi and

Kilham, 2001; Davies et al 2009; Foster et al 2011; Brauer et al 2012), by placing such a strong emphasis on resource availability, we may be missing the important effects that heterogeneity in other environmental gradients can have on metacommunity dynamics, specifically those that physiologically constrain species occurrences such as temperature (Prophet 1963; Branstrator et al 2013), moisture (Chisholm et al 2010), salinity (Nielsen et al 2003), and pH (Korosi and Smol 2011). These types of gradients are what often constitute species niches and drive species sorting in natural communities. Therefore, the differences, or contrasts, between habitat patches along these environmental gradients as well as the relative abundances of different types of habitat patches can have large implications for metacommunity composition and biodiversity (Biswas and Wagner 2012).

The salinization of inland freshwater systems is a global issue (Rengasamy, 2006) that is driven by a variety of processes, many of which are directly or indirectly connected to anthropogenic disturbances. Increased crop irrigation in arid regions is responsible for drawing up saline groundwater that is contaminating agricultural fields and draining into nearby waterbodies (Barica 1972). The use of road salts has increased the salinity of streams and lakes, making them toxic for native biota (Likens and Buso 2010; Corsi et al. 2010). However, the primary contributor to global salinization is changes in regional climate. As the climate of a region becomes more arid, its waterbodies become more saline as evaporation exceeds precipitation, and such changes have already been observed in lakes across the globe (Sereda et al. 2011; Aladin et al. 2009). Salinization severely decreases water quality (Williams et al 1990) and local biodiversity (Kang and King, 2012), making it one of the greatest threats to freshwater

ecosystems. While the impacts of salinity have been well-studied at the local-scale (Hammer 1986; Moss 1994; Williams 1998), very little is known about how the relative abundance and degree of environmental contrast between freshwater and salt water habitats across a landscape will influence metacommunity structure and biodiversity.

The Nebraska Sandhills are a large grass-stabilized dune system covering approximately 50,000 km^2 of central and western Nebraska. The Nebraska Sandhills region is overlain by a climate gradient in which precipitation decreases from east to west (Bleed and Flowerday 1998). This climate gradient has yielded a longitudinal environmental heterogeneity gradient in which the abundance of saline waterbodies increases westwards as evaporation exceeds precipitation (Bennett et al 2007; Mausbach and Dzialowski 2017). Mausbach and Dzialowski (2017) and Mausbach (Chapter 2) found that this westward increase in environmental heterogeneity corresponded with a decrease in the alpha and gamma richness of crustaceans from freshwater wetlands. Mausbach and Dzialowski (2017) hypothesized that the decrease in freshwater biodiversity may be influenced by a combination of the westward decline in freshwater wetland abundance and the increase in environmental contrasts between freshwater and saline wetlands.

Here I investigated how the interaction of environmental contrast between habitat patches and the spatial heterogeneity of those habitat patches within a landscape influence community and metacommunity composition and biodiversity in experimental mesocosms (Fig. 1). I hypothesized that freshwater alpha richness and metacommunity gamma richness would decrease as the relative abundance of freshwater patches decreased, because fewer freshwater patches would be available to provide rescue effects

(Loreau and Mouquet 1999; Leibold et al 2004) to mitigate stochastic extinctions (Fig. 1). I hypothesized that this trend in freshwater alpha and metacommunity gamma richness would be amplified with an increase in environmental contrast, because fewer species will be shared between high-contrasting habitat patches further reducing the chances of rescue effects occurring, thus increasing the risk of local and regional extinctions of freshwater species. I hypothesized that the species composition of the freshwater mesocosms would differ along the relative abundance gradient, because in metacommunities dominated by saline mesocosms, the freshwater mesocosms will frequently receive halotolerant species from the neighboring saline mesocosms. I did not expect to see any effect of spatial heterogeneity on the community composition or biodiversity of the saline mesocosms, because the local effects of salinity as an environmental filter will be stronger than the regional effects, and the species richness of the saline mesocosm will be too low for patterns to emerge.

Methods

Experimental Design

I combined two heterogeneity gradient treatments into a full-factorial design, so that both the low-contrast and high-contrast metacommunities consisted of freshwater:salinity mesocosm relative abundances of 3:1, 2:2, and 1:3 (Fig 1). Each metacommunity treatment contained four replicates, totaling 96 mesocosms. The experiment was conducted in an indoor wet lab at Oklahoma State University. Due to logistical constraints, the experiment was divided into two blocks that were conducted at different times. Each of the blocks contained 2 replicates of all the treatments. Conductivities of

the freshwater, low-salinity, and high-salinity treatments were 0.508 mS/cm (SD ± 0.036), 4.121 mS/cm (SD ± 0.196), and 10.279 mS/cm (SD ± 0.374) respectively. These levels were based on the conductivity levels from field surveys in which there were observable declines in species richness along a conductivity gradient (Mausbach and Dzialowski, 2017). For example, Mausbach and Dzialowski (2017) found 28 species occurring in waters near 0.5 mS/cm, 11 species occurring in waters near 4 mS/cm, and 6 species occurring in waters near 10 mS/cm. The mesocosms consisted of 75L white polyethylene containers and were filled with 50 L of dechlorinated water. The saline treatments were created by adding salts that were evaporated from water collected from Lakeside Lake, a saline lake in the Nebraska Sandhills. The ionic composition of inland saline waters varies considerably geographically, and branchiopod crustaceans have been reported to become locally adapted to geographically distinct saline solutions (Bowen et al 1985; Martínez-Jerónimo and Espinosa-Chávez 2005); therefore, it was important that I used salts from the Sandhills region for this experiment. For the first block, the water temperature varied throughout the room by less than 2°C averaging 20.65 °C (SD ± 0.68). The second block started with a similar temperature to the first block; however, from weeks 6 to 10 of Block 2, the temperature of the lab decreased to an average of 16.89°C (SD ± 0.65).

I inoculated the freshwater and salt water mesocosms with microbes to provide a naturally diverse assemblage of food resources for the cladocerans. The freshwater and saltwater microbes were obtained by soaking 500mL of sediment from freshwater and saline wetlands respectively in buckets filled with 16L of dechlorinated water. Fluorescent lights were placed over the buckets, and they were aerated with bubblers for

five days to encourage rapid growth. After 2 days of soaking, the sediment and any zooplankton that hatched were filtered out of the solutions through a 45 μm sieve. Each mesocosm then received 1L of the appropriate freshwater or saline microbial solution (both low- and high-salinity mesocosms received the same saline microbial solution). Fluorescent lights were placed above the mesocosms for a 16:8 day/night cycle. The microbial communities were given two weeks (Weeks 0 and 1) to grow in the mesocosms before the cladocerans were added. Microbial densities were not measured, however, there were visual increases in turbidity that decreased after the cladocerans were added. Seventeen lab-cultured cladoceran species were used to form the metacommunities (Table 1). To determine which species were added to each local condition (i.e. freshwater, low-salinity, or high-salinity), I exposed 10 individuals of each species to freshwater, low-salinity, and high-salinity conditions for 48 hours. If mortality was less than 50%, then five individuals of varying ages of that species were added to each mesocosm with that local condition. I did not control for the colonists ages, because I wanted to introduce some level of variation so that all the mesocosms with the same local conditions in a metacommunity would still have the same number and types of species, but not be completely identical (Grainger and Gilbert 2016). The freshwater mesocosms were inoculated with 16 species, the low-salinity mesocosms were inoculated with 5 species, and the high-salinity mesocosms were inoculated with 3 species (Table 1). The cladocerans were given two weeks (Weeks 2 and 3) to establish in the mesocosms.

I collected zooplankton samples from each mesocosm on Week 4 prior to the first dispersal event to assess the starting community compositions. Zooplankton samples were collected using a depth-integrated vertical PVC sampler with which I collected 8,

480mL grab samples from throughout the mesocosm that were combined into a single sample and preserved in 95% ethanol. These samples were collected at the beginning (Week 4) and end (Week 10) of the experiment. The zooplankton were enumerated and identified to species using subsamples consisting of a minimum of 250 individuals. The entire sample was than surveyed for rare species. I did not use rarefaction curves to estimate species richness because I controlled how many species were introduced to the metacommunity treatments; therefore, I knew what the total possible species richness could be for a mesocosm, and a rarefaction curve may have overestimated the species richness in some treatments (Resetarits et al. 2018).

Dispersal events between mesocosms within metacommunity treatments occurred weekly starting on Week 4 and ending on Week 9 (Fig. 1). I stirred the mesocosms to homogenize cladoceran distributions and then used a depth-integrated PVC-sampler to collect cladocerans for the dispersal events. The dispersal samples represented 4.8% of the total volume of the mesocosm (Howeth and Leibold 2010), so I collected 3 sets of 5, 480mL grabs (~2.5L total) to disperse to each neighboring community within the metacommunity treatment. Each mesocosm had a designated jar within which its immigrants were deposited until the immigrants were ready to be dispensed into the mesocosm. To avoid dispersing new colonists, I waited until I collected the dispersal samples from all the mesocosms within the given metacommunity before emptying the contents of the jars into their respective mesocosms. Since some colonists were moved from low- or high-salinity mesocosms, they were thoroughly rinsed with dechlorinated water prior to being introduced to freshwater mesocosms to avoid increasing the salinity of the freshwater mesocosms.

Statistical Analyses

I calculated the alpha richness of each habitat type (i.e. freshwater, low-salinity, or high-salinity) for the metacommunity treatments by averaging the alpha richness of environmentally equivalent mesocosms within the metacommunity replicates so that the data were balanced for analysis. For example, for the freshwater alpha richness of the freshwater-dominated metacommunity treatments, I averaged the alpha richness of the freshwater mesocosms within each individual metacommunity replicate, so there were four (one per metacommunity replicate) rather than twelve (three freshwater communities per metacommunity replicate) freshwater alpha richness data points. I used a two-way analysis of variance (ANOVA) to test the independent and interactive effects of environmental contrast and spatial heterogeneity on alpha richness of freshwater communities. I then performed two, one-way ANOVAs to compare the alpha richness of low-salinity and high-salinity communities along the spatial heterogeneity gradient. I used a two-way ANOVA to test the independent and interactive effects of environmental contrast and spatial heterogeneity on the gamma richness of the metacommunities. The ANOVA and *post hoc* analyses were conducted using SigmaStat 3.5.

I used principal response curves (PRC) (van den Brink et al. 2008) in CANOCO 5.04 to assess differences in community composition across metacommunity treatments. Principal response curves are beneficial for studying temporal changes in species composition across treatments over other ordination techniques because they show changes in community composition relative to a baseline treatment, which is designated by the user (Dee et al. 2016). Additionally, PRCs are easy to interpret as the x-axis

represents time and the y-axis represents community variation, so communities with response curves that follow similar trajectories along the y-axis are more similar than communities with response curves that diverge along the y-axis (Dee et al. 2016). I used a PRC to assess general species associations between freshwater, low-salinity, and high-salinity environments from environmental contrast and spatial heterogeneity metacommunity treatments. I then constructed individual PRCs for each salinity level to focus on differences across environmental contrast and spatial heterogeneity treatments. Since the low-salinity and high-salinity mesocosms were restricted to their respective environmental contrast treatments, their PRCs only compared differences along the spatial heterogeneity gradient. I also used a PRC to identify differences in metacommunity composition across the spatial heterogeneity and environmental contrast gradients.

Results

Spatial heterogeneity had diverse effects on the alpha and gamma richness of the different metacommunity treatments. Spatial heterogeneity had a significant effect on the alpha richness of the freshwater communities across both low- and high-contrast treatments (Fig. 2; Two-way ANOVA: $F_{2,23} = 4.937$, $P = 0.02$) with freshwater communities from saline-dominated metacommunities supporting significantly fewer species than those from the other metacommunity treatments (Fig. 2; *post hoc* Tukey Test, $P < 0.05$). Environmental contrast had no effect on freshwater alpha richness (Two-way ANOVA: $F_{1,23} = 1.505$, $P = 0.24$), and there was not a significant interaction between environmental contrast and spatial heterogeneity (Two-way ANOVA: $F_{2,23} =$

0.895, P = 0.90). Spatial heterogeneity had no effect on the alpha richness of low-salinity (One-way ANOVA: $F_{2,23}$ = 0.111, P = 0.90) or high-salinity (One-way ANOVA: $F_{2,23}$ = 0.011, P = 0.99) communities (Table 2). Gamma richness exhibited the same pattern as freshwater alpha richness (Fig. 3; Two-way ANOVA: spatial heterogeneity $F_{2,23}$ = 6.379, P = 0.008; environmental contrast $F_{1,23}$ = 0.0, P = 1.0; spatial heterogeneity*environmental contrast $F_{2,23}$ = 0.864, P = 0.44) with the saline-dominated metacommunities supporting significantly fewer species than the freshwater-dominated and the half freshwater/half saline metacommunities (Fig. 3; *post hoc* Tukey Test: P < 0.05).

There was a significant treatment effect on community composition (Fig. 4; PRC Axis 1 = 63.9% variance explained, F = 157, P = 0.001). The low- and high-salinity communities were characterized by high abundances of *Moina macrocopa*, *Moina hutchinsoni*, and *Daphnia similis*, whereas, freshwater communities typically consisted of *Scaphaloberis mucronata*, *Daphnia pulex*, *Simocephalus serrulatus*, *Ceriodaphnia dubia*, *Alona setulosa*, *Simocephalus vetulus*, and *Chydorus sphaericus* (Fig. 4). Freshwater communities diverged along the environmental contrast gradient (Fig. 5a; PRC Axis 1 = 13.3% variance explained, F = 6.0, P = 0.05). Freshwater communities from the low-contrast metacommunity treatments supported higher densities of *Alona salina*, *Daphnia similis*, and *Macrothrix rosea* than the high-contrast freshwater communities, indicating that species were more successful at dispersing between freshwater and saline communities in low-contrast metacommunities (Fig. 5a). High-contrast freshwater communities were dominated by freshwater taxa, indicating little successful immigration of halophilic taxa from neighboring high-salinity communities (Fig. 5a). There was no

effect of the spatial heterogeneity gradient on low- or high-salinity community compositions (Low-salinity PRC Axis 1 = 6.2% variance explained, F = 1.6, P = 0.68; High-salinity PRC Axis 1 = 8.8%, F = 2.0, P = 0.50). Metacommunity composition corresponded with the environmental contrast and spatial heterogeneity gradients (Fig. 5b; PRC Axis 1 = 19.5% variance explained, F = 22.4, P = 0.002) with metacommunities that contained more saline communities at higher salinities supporting higher abundances of halophilic taxa such as *D. similis*, *M. hutchinsoni*, and *M. macrocopa* (Fig. 5b). *Moina micrura* and *Oxyurella braevicaudata* were only found in freshwater mesocosms at the beginning of the experiment and rarely detected at the end of the experiment across all the treatments, which is why their species scores are near 0 on the species response diagrams for all of the PRC's.

Discussion

My findings indicate that the spatial heterogeneity of saline and freshwater patches affects biodiversity at local and regional scales. The influence of salinity on freshwater communities has been studied extensively (Hammer 1986; Williams 1998; James et al 2003; Thompson and Shurin 2012); however, these studies have focused on the effects of salinity at the local scale and did not consider the regional scale. Thompson and Shurin (2012) incorporated the regional species pool into their study and tested the effects of warming and salinization on freshwater zooplankton communities under the umbrella of future climate change. However, Thomson and Shurin (2012) utilized an independent regional species pool that consisted of zooplankton collected from regional lakes rather than a regional species pool that was derived from their experimental units,

so their findings were still limited to the effects of salinity at the local level. This is the first study to test how the relative abundance and the salinity of saline patches in experimental metacommunities influenced biodiversity and community composition at local and regional scales.

As expected, salinity served as a strong local environmental filter with low- and high-salinity communities being structured more by local conditions than regional processes. There was evidence of freshwater influences on low-salinity communities in the form of providing *Macrothrix rosea* immigrants to some of the low-salinity mesocosms; however, this only occurred in a few mesocosms, which is why it is not obvious in the PRC (Fig. 4). There were also occasional detections of other freshwater species in both low- and high-salinity mesocosms; however, the densities were very low, and not indicative of successful colonization. Freshwater communities were influenced by regional processes in that low and high-contrast metacommunities produced compositionally different freshwater communities (Fig. 5a). As expected, the low-contrast metacommunity allowed for more dispersal between the freshwater and saline communities. Freshwater communities in low-contrast metacommunities supported high abundances of *Alona salina* and *Daphnia similis* which were prominent in low-salinity communities, and a high abundance of *Macrothrix rosea*, which, as mentioned earlier, was detected in some low-salinity communities (Fig. 5a). Differences in environmental contrast may be more influential in driving population sizes of more tolerant species, but not in maintaining the populations of the more sensitive species that are driving species richness patterns.

As I hypothesized, freshwater alpha richness and metacommunity gamma richness declined as freshwater patches became less abundant within the metacommunity. However, I was expecting a gradual decrease in both alpha and gamma richness across all three spatial heterogeneity treatments (Fig 1), rather than a decrease in only the saline-dominated treatments. Nevertheless, these findings coincide with the field surveys from Mausbach and Dzialowski (2017) and Mausbach (Chapter 2) in that freshwater biodiversity declined at local and regional scales as freshwater habitats became less abundant across the landscape. Therefore, freshwater habitats serve as keystone communities (Mouquet et al 2013) in these natural (Mausbach and Dzialowski 2017; Chapter 2) and experimental metacommunities, as they disproportionately influence regional biodiversity and composition. Biodiversity-heterogeneity relationships are often positive (Cramer and Willig 2005), however, not all patches are equally species-rich so the type of patches constituting the heterogeneity gradient can be more important than the actual presence of heterogeneity (Chisholm et al 2010; Mouquet et al 2013). For example, Ptacnik et al (2010) found that the local richness of phytoplankton from Scandinavian lakes could not be predicted solely based on the local conditions of the lakes, but rather in conjunction with the abundance of environmentally similar neighboring lakes across the landscape. Thus, it is important that when conserving natural ecosystems, multiple, species-rich patches are being conserved throughout the landscape.

Mesocosm experiments are simplistic relative to natural systems, but they are powerful tools for studying the mechanisms structuring communities and metacommunities. However, there are some aspects of natural systems that are difficult to simulate in experimental mesocosms, particularly dispersal. The rapid shifts from

freshwater to saline or saline to freshwater environments in the high-contrast metacommunities was too physiologically strenuous to permit successful immigration for most species that would normally be able to live in both environments (Mausbach and Dzialowski 2017). In natural systems, zooplankton dispersal occurs via dormant egg stages that will hatch when exposed to the appropriate environmental cues (Brendonck and De Meester 2003), and changes in salinity are likely to occur over hours or days rather than within seconds, so species in natural systems have more time to acclimate to environmental changes and may therefore be present across a wider range of environmental conditions. A lower contrast treatment around 2mS/cm may have been more appropriate in this type of experiment as it is less physiologically demanding for many species, but still limiting enough for the more sensitive freshwater species to yield differences in species richness.

Species richness and community composition patterns will differ depending on the environmental gradient being tested as some gradients are more physiologically restrictive than others. Salinity is an ideal gradient from which to study metacommunity dynamics, because it influences a variety of water quality parameters and serves as a strong environmental filter for species across many diverse taxonomic groups (Hammer 1986; Williams 1998). However, salinity gradients are only useful when the constituents of the saline solutions are ecologically relevant for the region from which the test subjects originated. Numerous studies that have conducted mesocosm or lifetable experiments to test the effects of salinization on community structure, mortality, or population growth have used ecologically irrelevant saline solutions such as pure NaCl solutions or commercial marine salts (Nielsen et al 2003; Sarma et al 2006; Thompson and Shurin

2012; Santangelo et al 2013). Different saline solutions will have different effects on species survival rates (Mount et al 1997), and marine salts have been found to be more toxic to halophilic taxa than the natural brines from which they occur (Martínez-Jerónimo and Espinosa-Chávez, 2005). Therefore, it is important that the appropriate salts are being used when asking questions about the effects of salinization on native ecosystems, because ecologically irrelevant salts will likely have a stronger negative effect than local naturally occurring salts.

While metacommunity ecology emphasizes the interplay between local and regional processes (Leibold et al 2004), too little is known about how environmentally different patches influence other communities through dispersal, particularly in metacommunities in which patches have disproportionate effects on regional biodiversity and metacommunity dynamics. Biodiversity-heterogeneity relationships described by the biodiversity-heterogeneity hypothesis (Cramer and Willig 2005) and area-heterogeneity tradeoff theory (Allouche et al 2012) often revolve around the number of different habitat types across the landscape, assuming the different patches are contributing equally to the regional species pool. However, habitat identity may be more important than habitat diversity. This study is an example of how the reduction of a single habitat type (i.e. freshwater patches) across the landscape can indirectly lead to local and regional species extinctions in metacommunities where that habitat type has a disproportionate effect on metacommunity dynamics.

This study is the first to empirically test the effects of environmental contrasts on local and regional biodiversity in a metacommunity context. While I did not find an effect of environmental contrast on biodiversity, habitat patches in low-contrast

metacommunities exhibited higher dispersal success as was indicated by the population abundances of select species that were tolerant of freshwater and low-salinity conditions. The influence of diverse habitat types on neighboring patches need to be explored in more depth using different heterogeneity patterns, environmental gradients, and taxonomic groups. Ecological disturbances and environmental changes are increasing across natural landscapes, and conservation practices need to be cognizant of the importance of maintaining multiple high-quality habitat patches to conserve local and regional biodiversity.

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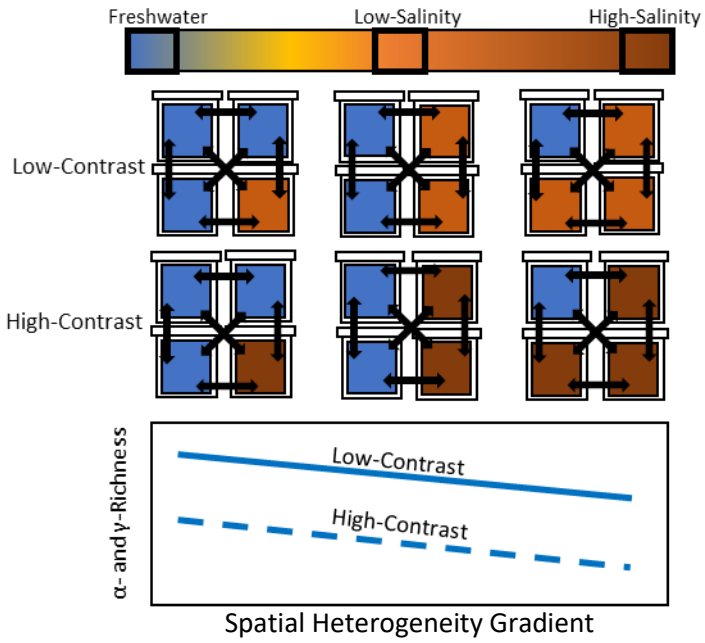

Figure 1. A visual diagram of the mesocosm experimental treatments. As indicated by the gradient, blue represents freshwater (~0.508 mS/cm), light brown represents low-salinity (~4.121 mS/cm), and dark brown represents high-salinity (~10.279 mS/cm). The black arrows represent dispersal pathways between mesocosms within a single metacommunity. The bottom figure is a visual representation of our hypotheses regarding the responses of α- and γ-richness of freshwater mesocosms to the spatial heterogeneity gradient at low and high contrasts.

Table 1. Lists of species that were added to mesocosms of each habitat type. To determine which species were added to each habitat type, ten individuals of each species were exposed to freshwater, low-salinity, and high-salinity conditions for 48 hours. They were then added to each habitat type for which mortality was less than 50%.

Species	Freshwater (0.5mS/cm)	Low-Salinity (4.0 mS/cm)	High-Salinity (10.0 mS/cm)
Moina hutchinsoni		X	X
Moina macrocopa	X	X	X
Moin micrura	X		
Daphnia pulex	X		
Daphnia magna	X		
Daphnia similis	X	X	X
Scaphaloberis mucronata	X		
Ceriodaphnia dubia	X		
Simocephalus vetulus	X		
Simocephalus serrulatus	X		
Macrothrix rosea	X		
Lathonura rectirostris	X		
Alona setulosa	X	X	
Alona salina	X	X	
Pleuroxus denticulatus	X		
Chydorus sphaericus	X		
Oxyurella braevicaudata	X		

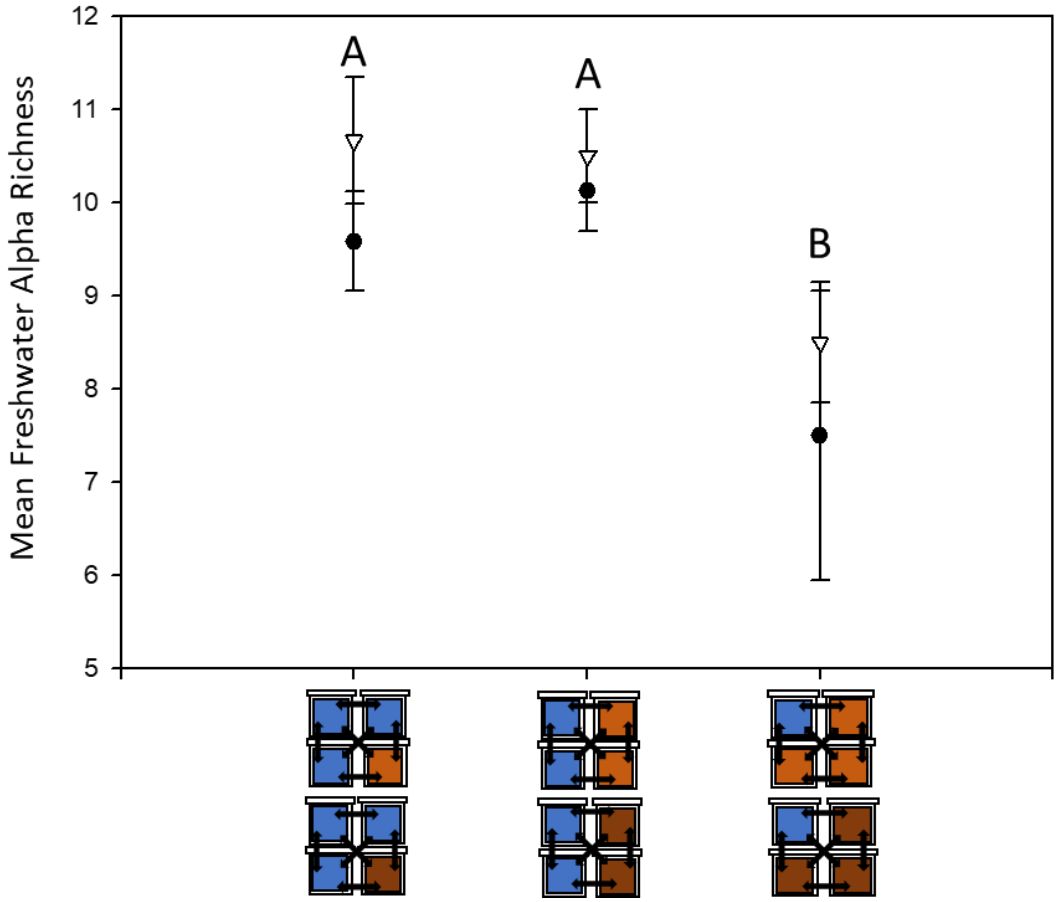

Figure 2. Mean alpha richness of freshwater communities from low-contrast (upside-down triangle) and high-contrast (circle) metacommunity treatments along the spatial heterogeneity gradient at the end of the experiment. Two-Way ANOVA was used to compare effects of environmental contrast and spatial heterogeneity on freshwater alpha richness. Spatial heterogeneity had a significant effect ($p = 0.02$) on alpha richness and there was no effect of environmental contrast ($p = 0.24$), therefore, the lettering A-B is used to designate significant differences between spatial heterogeneity treatments. Error bars are standard error.

Table 2. One-way ANOVA results comparing alpha richness of the low- and high-salinity treatments across the spatial heterogeneity gradient.

Salinity	Spatial Heterogeneity	Mean	Std. Error	DF	F-statistic	P-value
Low	Freshwater-dominated	4.5	0.87	2,23	0.011	0.99
	Half Fresh/ Half Saline	4.5	0.46			
	Saline-dominated	4.42	0.38			
High	Freshwater-dominated	3.75	0.63	2,23	0.11	0.9
	Half Fresh/ Half Saline	3.5	0.42			
	Saline-dominated	3.42	0.36			

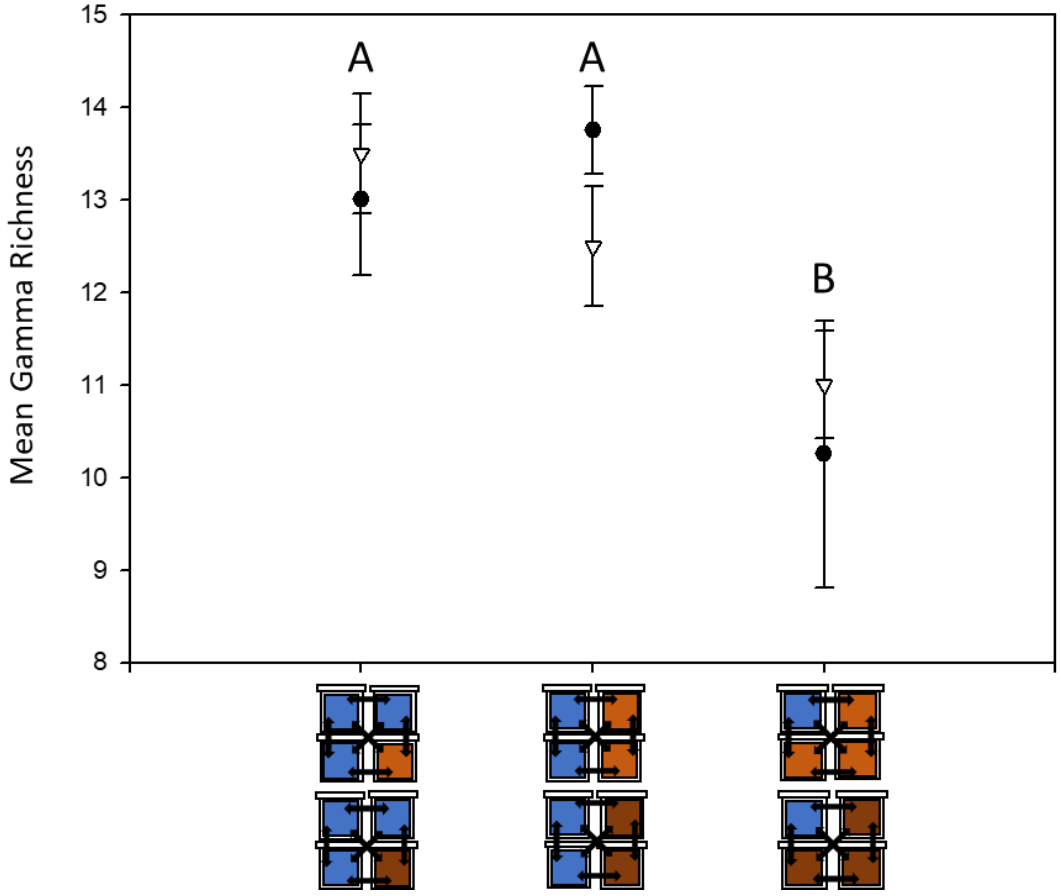

Figure 3. Mean gamma richness from low-contrast (upside-down triangle) and high-contrast (circle) metacommunity treatments along the spatial heterogeneity gradient at the end of the experiment. Two-Way ANOVA was used to compare effects of environmental contrast and spatial heterogeneity on gamma richness. Spatial heterogeneity had a significant effect ($p = 0.008$) on alpha richness and there was no effect of environmental contrast ($p = 1.0$), therefore, the lettering A-B is used to designate significant differences between spatial heterogeneity treatments. Error bars are standard error.

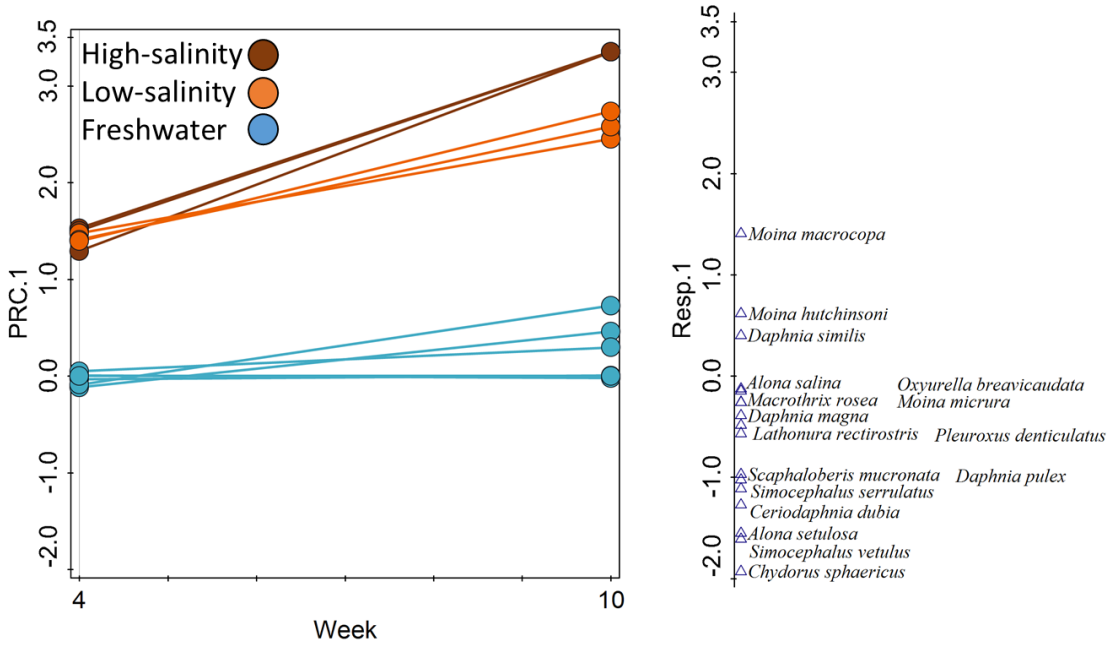

Figure 4. Principal response curves displaying general species-environmental associations in freshwater, low-salinity, and high-salinity communities across metacommunity treatments. The freshwater communities from the low-contrast freshwater-dominated metacommunity treatment were used as the baseline. PRC Axis 1 = 63.9% variance explained, F = 157, P = 0.001. There were no significant differences within the low-salinity or high-salinity communities along the spatial heterogeneity gradient (low-salinity P = 0.68; high-salinity P = 0.50), so individual PRC diagrams for those treatments are not shown.

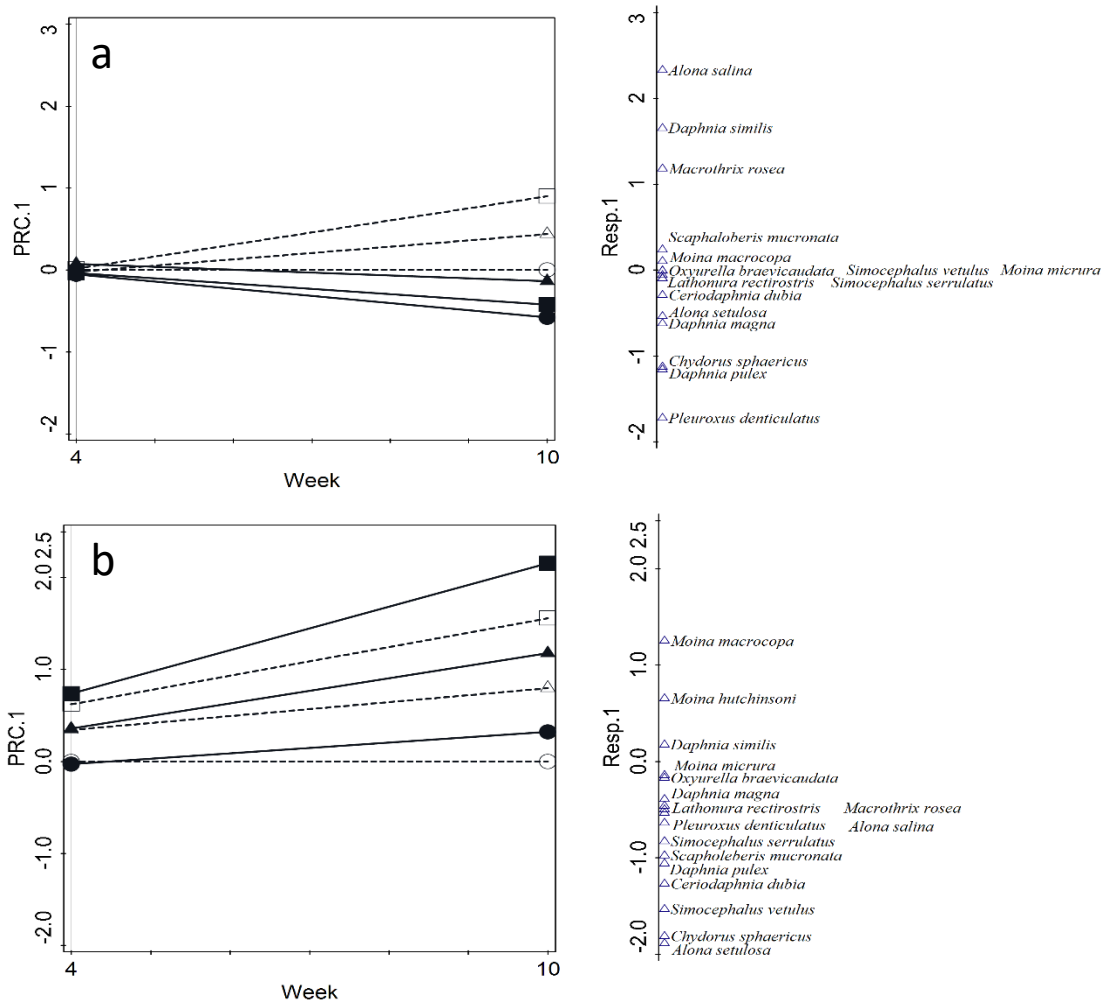

Figure 5. Principal response curves comparing community and metacommunity compositions across the environmental contrast and spatial heterogeneity gradients. A) Local freshwater communities (PRC axis 1 = 13.3% variance explained, P = 0.05). B) Metacommunities (PRC axis 1 = 19.5% variance explained, P = 0.002). The freshwater-dominated, low-contrast metacommunity treatment was used for the baseline for both A and B. Symbology: Low-contrast (white shapes, dashed lines), High-contrast (black shapes, solid lines); freshwater-dominated (circle), half freshwater/half saline (triangle), and saline-dominated (square).

CHAPTER IV

IT TAKES A METACOMMUNITY: DISPERSAL MITIGATES BIOLOGICALLY-INDUCED DISTURBANCES IN HETEROGENEOUS METACOMMUNITIES

Abstract

The global increase in anthropogenic disturbances has introduced patterns of heterogeneity across many landscapes. Local disturbances are often studied from the perspective of the local habitat patch, where their direct effects on local community composition are more evident. However, local disturbances can have regional implications when connected to other patches within a metacommunity. Invasive species provide a unique opportunity for studying the influence of biologically-induced disturbances on metacommunity dynamics, because invasive species are influential in structuring local communities and have a propensity for dispersing across landscapes, producing gradients in disturbance intensity. Furthermore, invasive species populations can become locally extinct from population crashes, allowing communities to recover from disturbances. The invasive zebra mussel, *Dreissena polymorpha*, is wide-spread throughout the eastern United States and is having negative impacts on zooplankton

communities. Using zebra mussels to induce disturbances in mesocosm zooplankton communities, I tested how the relative abundance of disturbed and undisturbed habitat patches within a metacommunity influenced community and metacommunity biodiversity and composition, and how those communities and metacommunities recovered after the invader was removed. The disturbance intensity gradient consisted of metacommunities comprised of 3 communities in which 0, 1, 2, or 3 of the communities were invaded by zebra mussels. Two isolated treatments were used to test the influence of the disturbance and the lack-there-of on local communities not connected to metacommunity networks. The zebra mussels negatively affected pelagic zooplankton species richness, while facilitating the population growth of littoral species. Undisturbed communities mitigated disturbance-induced local and regional pelagic species loss in heterogeneous metacommunities, while the fully-disturbed metacommunity experienced significant losses of pelagic zooplankton at local and regional scales. Disturbed communities influenced undisturbed communities in heterogeneous metacommunities by increasing the densities of littoral zooplankton species. The communities and metacommunities did not recover to non-disturbance conditions after the zebra mussels were removed and exhibited non-intuitive patterns in which the condition of some treatments worsened, which may have been caused by low-quality algal resources in the mesocosms. Overall, these results highlight the important effects that undisturbed habitat patches can have on mitigating species loss in locally disturbed patches and maintaining regional biodiversity in heterogeneous metacommunities, and the severe impacts that disturbances can have on local and regional scales in fully-disturbed metacommunities.

Introduction

The global increase in anthropogenic disturbances has introduced patterns of heterogeneity across many landscapes in the form of habitat loss, the spread of invasive species, pollution, and urbanization (Hamer and Hill 2000; Woods et al. 2016; Shackelford et al. 2017; Shackelford et al. 2018). These disturbances are often studied from the local perspective, because they have strong species-sorting effects on communities that often lead to local extinctions. However, disturbed communities are often connected to neighboring communities through dispersal networks, forming metacommunities. Therefore, the effects of these localized disturbances can be extended to neighboring communities and in turn the regional species pool (Strayer et al. 2004; Sousa et al. 2009; terHorst and Dudgeon 2009; Howeth and Leibold 2013; Woods et al. 2016). Alternatively, the regional species pool can mitigate the impacts of disturbance on local communities by supplying colonists to reduce extinction rates or quicken the post-disturbance recovery through mass or rescue effects (Mouqet and Loreau 2002; Leibold and Nornberg 2004; Cramer et al. 2008; Shackelford et al. 2017; Shackelford et al. 2018).

The dynamics between the disturbed and undisturbed communities within a metacommunity will depend on the relative abundance of each habitat type within the metacommunity, the connectivity of those habitat types, and the effect of the disturbance on the local patches (Cramer et al. 2008; Howeth and Leibold 2013). A metacommunity dominated by disturbed patches will be less likely to mitigate the local effects of disturbances than a metacommunity consisting of less disturbed patches, because there will be fewer undisturbed source patches to provide colonists to the disturbed patches. The influence of disturbance on local and regional scales is often context dependent and

may facilitate some species while impeding others. A highly disturbed metacommunity is more likely to lose species from the regional species pool, because there is a greater chance that patches containing regionally rare or uncommon species will be disturbed, which may lead to the regional extinctions of those species (Wright et al. 2004; Smith et al. 2009; Woods et al. 2016). Alternatively, introducing disturbance into a metacommunity could bolster regional biodiversity by altering local patches in such a way that the disturbed patches are beneficial to species that were previously unable to utilize those patches, which can include regionally uncommon or rare species (Jones et al. 1997; Wright et al. 2004; Rodriguez 2006).

I was interested in how the relative abundance of disturbed and undisturbed habitat patches within a metacommunity influenced the local biodiversity and composition of patches as well as the biodiversity and composition of the metacommunities. Invasive species provide a unique opportunity to study metacommunity dynamics in regards to ecological disturbances and landscape heterogeneity, because invasive species are often very influential in structuring the communities in which they invade (Mack et al. 2000; Wigginton et al. 2014), they can introduce or reduce biologically-driven heterogeneity to the landscape (Beisner et al. 2003; Rodriguez 2006; Boughton and Boughton 2014), they spread rapidly across landscapes (Havel et al. 2015), and they can be removed or experience population crashes, thus allowing the invaded system the opportunity to recover from the disturbance. Furthermore, the impacts that invader-induced heterogeneity can have on regional scales is largely unknown (Melbourne et al. 2007). I addressed these questions using the invasive zebra mussel, *Dreissena polymorpha*, which is highly invasive in

North American lakes, rivers, and reservoirs (Higgins and Vander Zanden 2010). Zebra mussels have high filtration rates that can cause trophic cascades if they outcompete pelagic zooplankton, which are an important prey resource for many predators (Strayer et al. 2004). Alternatively, by reducing phytoplankton biomass, zebra mussels encourage the growth of littoral vegetation and periphyton by increasing light penetration through the water column and reducing competition for dissolved nutrients (Higgins and Vander Zanden 2010), which can facilitate the increase in littoral zooplankton species richness and biomass (Dzialowski, 2013; Sinclair et al. 2015).

The objective of my study was to determine how increases in the relative abundance of invader-induced disturbances influenced biodiversity and species composition at community and metacommunity scales in experimental mesocosms. I was specifically interested in how species composition and diversity would change along a gradient of disturbance-intensity in which the proportion of disturbed (presence/absence of zebra mussels) communities within the metacommunity treatment increased from a homogeneous undisturbed metacommunity (i.e. no mesocosms invaded) to a homogeneous fully-disturbed metacommunity (i.e. all mesocosms invaded). Due to the temporal nature of ecological disturbances and invasions, I also wanted to test how metacommunity disturbance intensity influenced the recovery of disturbed communities after the zebra mussels were removed.

I hypothesized that the alpha diversity of the disturbed and undisturbed communities would decrease as the relative abundance of disturbed communities within the metacommunity increased, because there would be fewer undisturbed patches to mitigate species loss in disturbed patches and prevent stochastic extinctions in

undisturbed patches. We expected gamma richness to be highest in the heterogeneous metacommunities, because the disturbed and undisturbed patches would support different species, promoting a higher beta-diversity than homogeneous metacommunities. We hypothesized that community composition between disturbed communities across metacommunity treatments will be more similar to each other, than the similarity in community composition between undisturbed communities across treatments, because the disturbed communities will be structured by a strong local filter, whereas, the undisturbed communities are going to be structured more by stochastic processes and possibly their neighboring disturbed communities. Therefore, we also hypothesized that metacommunity species composition will follow the disturbance-intensity gradient in which metacommunities with more disturbed patches will have more similar species compositions. Assuming that my hypotheses are supported, I expect that, after I remove the zebra mussels for the post-disturbance phase of the study, dispersal from undisturbed communities, which is contingent upon them still being intact, will cause the disturbed communities from heterogeneous metacommunities to gradually resemble undisturbed communities. Additionally, the species composition of the two heterogeneous metacommunities will begin to resemble the homogeneous undisturbed metacommunity as the local communities start to resemble undisturbed communities.

Methods

Experimental design

I conducted a twelve-week indoor mesocosm experiment in which I used zebra mussels to create a disturbance-intensity gradient to determine how biologically-induced

heterogeneity influenced metacommunity dynamics. I established four metacommunity treatments, where each metacommunity consisted of three 75L polyethylene mesocosms (communities) that were replicated in triplicate. These metacommunity treatments represented an accumulative disturbance gradient in which the proportion of communities with zebra mussels increased from a metacommunity with zero disturbed communities to a metacommunity in which all three of the communities were disturbed (Fig. 1). Here on, I refer to each metacommunity treatment by the number of disturbed communities within that metacommunity (i.e., Treatment 0, 1, 2, and 3). I also constructed two isolated treatments in which there was no dispersal that consisted of a single mesocosm replicated in triplicate where one treatment was disturbed and the other was not. These isolated treatments were compared to the metacommunity treatments so that I could account for the influence of dispersal on the alpha diversity of undisturbed and disturbed communities across the disturbance-intensity gradient. The whole experiment consisted of 42 mesocosms (4 metacommunity treatments x 3 communities per metacommunity x 3 replicates + 2 isolated treatments x 3 replicates = 42 mesocosms).

On June 19th, 2014, I added 8L of filtered (64 µm mesh) lake water collected from Boomer Lake, Stillwater, OK, to each mesocosm to inoculate them with algal and bacterial resources, and then I filled the remainder with de-chlorinated tap water (75 L total). Each mesocosm was covered with a fluorescent shop-light set for 16/8hr light/dark days. I added 100 µg/L of phosphorus to each mesocosm to encourage phytoplankton growth. On June 21st, I collected zooplankton from five permanent waterbodies within and around Stillwater, OK, in which zebra mussels had not been detected. I removed zooplankton predators, *Leptodora kindti* and *Chaoborus sp*. larvae, by washing the

zooplankton mixture through a 2mm mesh sieve into a 45 µm mesh sieve, and I then visually surveyed the material under a dissecting microscope to remove any predators that may have fallen through the larger mesh. Few predators were detected in the sieves and none were ever detected in the mesocosms throughout the experiment. Each mesocosm was inoculated with the zooplankton mixture so that the mean starting zooplankton densities in the mesocosms was 2.98 ± SE 0.65 individuals/L. I added 35 µg/L of phosphorus to each mesocosm weekly to replenish weekly phosphorus losses, which I estimated from a pilot study conducted two weeks prior to this experiment. Similar phosphorus loss rates have been estimated in other mesocosm studies (Hall et al. 2005; Schuler et al. 2017).

I added 18 zebra mussels (0.24 mussels/L) averaging 2.3 (SD ± 0.3) cm in length to each of the disturbed mesocosms five days after the zooplankton were added. Although mussel densities are difficult to simulate in mesocosms because they tend to aggregate in natural systems and are not evenly distributed throughout the water column, the density that I used is consistent with previous studies (Dzialowski 2013; Sinclair and Arnott 2015; Sinclair et al. 2015). Furthermore, Sinclair and Arnott (2015) tested the influence of zebra mussel densities and nutrient addition on mesocosm zooplankton communities, and they found that 0.25 zebra mussels/L had the same effect on zooplankton communities as 1.0 zebra mussels/L, so I was confident that our chosen density would have an observable ecological effect. After Week 6, I removed all the zebra mussels from the disturbed mesocosms and continued the study for six more weeks to determine how the post-disturbed communities and metacommunities responded to the zebra mussel removal.

For dispersal, each mesocosm received zooplankton from each of the two other mesocosms within the same metacommunity three times each week for twelve weeks. Our dispersal rate reflected a scenario in which each week 5% of the total mesocosm volume was dispersed to each other mesocosm within the treatment (Cohen and Shurin 2003; Howeth and Leibold 2010). I simulated dispersal by first stirring each mesocosm to homogenize the zooplankton distributions throughout the water column, and I then used a depth-integrated PVC sampler to extract 1.2L of water from the mesocosm. I washed the samples through a 64 μm mesh, returning the water to the mesocosm and then deposited the immigrating zooplankton into beakers designated for each receiving mesocosm. To avoid re-dispersing zooplankton between the mesocosms, I waited until I had collected zooplankton from all the mesocosms within the metacommunity prior to transferring the immigrant zooplankton mixtures into the designated mesocosms. To avoid excessive dispersal and contamination between mesocosms, I thoroughly rinsed all the equipment in between sampling each mesocosm. To account for any disturbances caused by the dispersal events, I used the dispersal protocol described above in the non-dispersal treatments, but I poured the zooplankton back into their resident mesocosms after they were extracted.

Sampling

I collected zooplankton and measured algal abundance from the mesocosms prior to introducing zebra mussels to the disturbance treatments and every three weeks thereafter. I collected zooplankton using a depth-integrated PVC sampler to extract 4L of gently stirred water from each mesocosm; each PVC sampler grab was 0.5L, therefore I

collected 8 water samples total from throughout each mesocosm. I washed the samples through a 64 µm mesh, returning the water back to the mesocosms, and I preserved the zooplankton in 95% ethanol for identification and enumeration. I counted and identified zooplankton to species using subsamples consisting of a minimum of 250 individuals. I measured algal abundance, because it is the primary resource for which zebra mussels and zooplankton compete. I measured the algal abundance of each mesocosm *in vivo* as relative fluorescence with a Turner Trilogy Fluorometer by collecting a 10mL water sample from just below the surface. I added de-chlorinated water to the mesocosms throughout the experiment to maintain water levels and ensure that any changes in zooplankton density or algal abundance were not influenced by changes in water volume.

Analytical methods

I compared algal abundance between disturbed and undisturbed mesocosms to test whether zebra mussels had a significant effect on algal abundance. I was only interested in the local effects that zebra mussels had on algal resources in disturbed communities; therefore, I grouped all the mesocosms into disturbed and undisturbed groups, ignoring the metacommunity and isolated treatment effects. I compared algal abundance between the disturbed and undisturbed mesocosms over time using a two-way repeated measures analysis of variance (RM ANOVA) and the Tukey method for *post hoc* pairwise interactions, using SigmaStat 3.5.

I assessed the influence of local disturbance (presence/absence of zebra mussels), metacommunity disturbance intensity (proportion of disturbed communities), and the role of dispersal on pelagic and littoral zooplankton alpha richness by performing two-way

RM ANOVA's. Since all the local conditions (disturbed or undisturbed) were not present in all the metacommunity treatments, I used community identity as the treatment factor in the RM ANOVA's in which communities were identified by their respective metacommunity treatments or if they were isolated treatments and whether they were locally disturbed or undisturbed. I acknowledge that the disturbed and undisturbed communities within the heterogeneous metacommunity treatments are not independent of each other, therefore, I was careful to not interpret them as such. I grouped the zooplankton into pelagic and littoral groups following Dzialowski (2013) and Sinclair et al. (2015), because zebra mussels negatively influence pelagic zooplankton species, while promoting littoral zooplankton species (Dzialowski 2013; Sinclair et al. 2015) (Table 1). Thus, if there is sufficient turnover from pelagic-dominated to littoral-dominated communities, I may not observe a change in alpha richness, even though species are being discriminately lost. I used the Tukey Method *post hoc* for pairwise interactions, so I could compare the alpha richness of the disturbed and undisturbed communities within and between the metacommunity treatments and the isolated treatments. I also tested how metacommunity disturbance-intensity affected the gamma richness of pelagic and littoral zooplankton by performing separate two-way RM ANOVA for both zooplankton groups. I used the Tukey Method *post hoc* for pairwise interactions. I performed all the analyses above separately for Weeks 1-6 (disturbance) and Weeks 6-12 (post-disturbance) datasets.

I used principal response curves (PRC) (van den Brink et al. 2008) in CANOCO 5.04 to assess community- and metacommunity-level differences in species composition within and between metacommunity treatments over time, as well as, to assess the

relative effect that dispersal had on disturbed and undisturbed communities. Principal response curves are beneficial for studying temporal changes in community composition across treatments over other ordination techniques, because they show changes in community composition relative to a baseline treatment, which is designated by the user (Dee et al. 2016). Additionally, PRC's are easy to interpret as the x-axis represents time and the y-axis represents community variation, so communities with response curves that follow similar trajectories along the y-axis are more similar than communities with response curves that diverge along the y-axis (Dee et al. 2016). For the community-level PRC's, I compared the disturbed and undisturbed communities within and between the metacommunity treatments to determine how disturbed and undisturbed communities differed along the disturbance-intensity gradient. To assess the role of dispersal and disturbance-intensity on disturbed and undisturbed communities, I constructed two PRC's: one exclusively for disturbed communities, including those from the isolated treatment, and the other exclusively for the undisturbed communities, including those from the isolated treatment. I then constructed PRC's to determine how the disturbance-intensity gradient affected metacommunity composition. I used Treatment 3 as the baseline for the metacommunity-level analyses, because all the mesocosms in Treatment 3 were exposed to the same ecological filter (zebra mussels), making them hypothetically less variable than Treatment 0, which was more open to stochastic processes. In the PRC's that assessed the influence of dispersal on community composition, I used the isolated treatments as the baselines, so that I could assess how dispersal and disturbance-intensity interacted to structure the undisturbed and disturbed communities. I performed

all the PRC analyses listed above separately for Week 1-6 (disturbance) and Week 6-12 (post-disturbance) datasets.

Results

Algal concentrations were reduced by more than half of their starting concentrations in both disturbed and undisturbed mesocosms within the first week of the experiment after zooplankton were added and continued to decrease until Week 3, after which they started to increase (Fig. 2). Beginning on Week 1 and throughout much of the study, disturbed mesocosms had significantly less algae than undisturbed mesocosms (Fig. 2; Two-way RM ANOVA: $F_{1,40} = 17.309$, $P < 0.001$). Following the removal of zebra mussels, algae concentrations increased in the disturbed mesocosms, reaching concentrations similar to the undisturbed mesocosms by end of the study (Fig. 2).

Disturbance

Pelagic alpha richness significantly declined within the first six weeks of the experiment in all disturbed and undisturbed communities across all metacommunity and isolated treatments (Fig. 3a; Two-way RM ANOVA: week $F_{2,68} = 273.32$, $P<0.001$). Community identity had a significant effect on pelagic alpha richness (Fig. 3a; Two-way RM ANOVA: treatment $F_{7,34} = 19.72$, $P<0.001$). Disturbance had a significant negative effect on alpha pelagic richness in isolated communities and communities from homogeneously disturbed metacommunities (Table 2; Tukey: $P \leq 0.05$). Disturbed communities from heterogeneous metacommunities contained significantly more pelagic species than isolated disturbed communities indicating that dispersal within

heterogeneous metacommunities has a positive effect on pelagic alpha richness (Table 2; Tukey: $P \leq 0.05$). However, dispersal had no effect on the pelagic richness of undisturbed communities in any of the metacommunity treatments (Table 2; Tukey: $P \leq 0.05$).

Littoral alpha richness significantly increased in all community treatments within the first six weeks (Fig. 3b; Two-way RM ANOVA: week $F_{2,40} = 30.27$, $P < 0.001$). Littoral alpha richness was not affected by metacommunity treatment or by dispersal (Fig. 3b; Two-way RM ANOVA: treatment $F_{3,20} = 0.13$, $P = 0.106$). It is important to note that the densities of these littoral species were too low for detection during the early stages of the experiment, so alpha richness may not have necessarily increased, but rather their populations eventually grew large enough to where they could be detected. Many littoral species, particularly those within the family Chydoriidae are less fecund than pelagic species, so their population growth rates are going to be slower (Thorp and Covich 2010).

Pelagic gamma richness significantly decreased in all the metacommunities over the first six weeks (Fig. 4a; Two-way RM ANOVA: week $F_{2,16} = 112.53$, $P < 0.001$). The homogeneously disturbed metacommunity supported significantly fewer pelagic species than the other metacommunity treatments, which did not differ from each other in pelagic gamma richness (Fig. 4a; Two-way RM ANOVA: treatment $F_{3,8} = 12.3$, $P = 0.002$; Table 3; Tukey: $P \leq 0.05$). Littoral gamma richness significantly increased in all the metacommunities over the first six weeks (Fig. 4b; Two-way RM ANOVA: week $F_{2,16} = 36.27$, $P <0.001$); however, there was no effect of metacommunity treatment on littoral gamma richness (Fig. 4b; Two-way RM ANOVA: treatment $F_{3,8} = 0.39$, $P = 0.762$).

Community identity had a significant effect on structuring local zooplankton communities (Fig. 5a; PRC axis 1, F = 27.9, P = 0.002, explained variation = 65.8%). Zooplankton communities were primarily driven by disturbance with littoral species being more abundant in disturbed communities and pelagic species being more abundant in undisturbed communities (Fig. 5a). While it appears that the disturbed communities differed more from each other based on metacommunity treatment than did the undisturbed communities, there was significantly more variation between the undisturbed communities across metacommunity treatments (Fig. 5b; PRC axis 1, F = 7.7, P = 0.05, explained variation = 63.6%) than there was between the disturbed communities (PRC axis 1, F = 4.9, P = 0.38, explained variation =45%). The variation in community composition between undisturbed communities across metacommunity treatments was primarily driven by the high abundance of littoral species and *Ceriodaphnia* in the undisturbed community from Treatment 2 (Fig. 5b). There was an interactive effect of dispersal and metacommunity heterogeneity on community composition of undisturbed communities as undisturbed communities from heterogeneous metacommunities (Treatments 1 and 2) differed from undisturbed communities that were not connected to disturbed communities (Treatment 0 and the isolated undisturbed treatment) by supporting higher densities of littoral species (Fig. 5b).

There was a significant effect of disturbance intensity on metacommunity composition (Fig. 6; PRC axis 1, F = 20.1, P = 0.002, explained variation = 70.3%). Littoral species were more abundant in disturbed metacommunities, whereas, pelagic species were more abundant in metacommunities with fewer or zero disturbed communities (Fig. 6). Metacommunity composition corresponded with the disturbance intensity gradient with

Treatments 0 and 3 being the most dissimilar. Although Treatments 1 and 2 were experimental intermediates between Treatments 0 and 3, they were compositionally more similar to Treatment 0 than Treatment 3 (Fig. 6).

Post-disturbance

Pelagic alpha richness did not change significantly overtime in any of the disturbed or undisturbed communities across isolated and metacommunity treatments after the zebra mussels were removed (Fig. 7a; Two-way RM ANOVA: week $F_{2,68} = 1.093$, $P = 0.341$). Metacommunity treatment continued to have a significant effect on pelagic alpha richness (Fig. 7a; Two-way RM ANOVA: treatment $F_{7,34} = 5.552$, $P<0.001$); however, the post-invasion differences in pelagic richness between disturbed and undisturbed communities within and between metacommunity treatments was less than when zebra mussels were present (Table 2 and 4).

Littoral alpha richness did not change significantly overtime in any of the disturbed or undisturbed communities across dispersal and metacommunity treatments after the zebra mussels were removed (Fig. 7b; Two-way RM ANOVA: week $F_{2,68} = 1.551$, $P = 0.219$). Littoral alpha richness was significantly influenced by metacommunity treatment and dispersal (Fig. 7b; Two-way RM ANOVA: treatment $F_{7,34} = 3.830$, $P = 0.004$) in which both the disturbed and undisturbed communities from Treatment 2 contained significantly more littoral species than the undisturbed isolated communities (Table 4).

Pelagic gamma richness did not change significantly overtime in any of the metacommunities for the remainder of the experiment (Fig. 8a; Two-way RM ANOVA:

week $F_{2,16}$ = 1.0, P = 0.390). Metacommunity Treatment 3 continued to exhibit a significantly lower pelagic gamma richness than the other three metacommunity treatments (Fig. 8a; Two-way RM ANOVA: treatment $F_{3,8}$ = 9.136, P = 0.006; Table 5, Tukey: P = 0.05). Littoral gamma richness did not change significantly overtime in any of the metacommunities for the remainder of the experiment (Fig. 8b; Two-way RM ANOVA: week $F_{2,16}$ = 3.444, P = 0.057); however, Treatment 2 had a significantly higher littoral gamma richness than Treatments 1 and 3 (Fig. 8b; Two-way RM ANOVA: treatment $F_{3,8}$ = 6.444, P = 0.0.16; Table 5, Tukey: P = 0.05).

Community identity continued to have a significant effect on structuring local zooplankton communities after zebra mussels had been removed (Fig. 9; PRC axis 1, F = 22.8, P = 0.001, explained variation = 60.5%); however, the final compositions of the two heterogeneous metacommunity treatments (Treatment 1 and 2) differed from the first half of the experiment (Fig. 9). By the end of the experiment, Treatment 1 was compositionally similar to Treatment 3, while Treatment 2 was compositionally similar to treatment 0 (Fig. 9). These compositional differences were driven by the abundances of a few select species. *Ceriodaphnia dubia*, *Lathonura rectirostris*, and *Bosmina longirostris* were highly abundant in Treatment 0 and 2 communities, while *Diaphanasoma bergei* was highly abundant in Treatment 1 and 3 communities (Fig. 9). Despite the significant post-disturbance differences in community composition across metacommunity treatments, when the communities were split into undisturbed and disturbed groups, I found no significant differences in species composition between metacommunity and isolated treatments (Disturbed: P = 0.796; Undisturbed: P = 0.860).

Disturbance intensity continued to have a significant effect on metacommunity composition after zebra mussels had been removed (Fig. 10; PRC axis 1, F = 19.6, P = 0.001, explained variation = 62.0%). However, metacommunity composition followed the same pattern as the local communities in that Treatments 0 and 2 were compositionally similar with high abundances of *Ceriodaphnia*, *Lathonura*, and *Bosmina* while Treatments 1 and 3 were compositionally similar with high abundances of *Diaphanasoma* (Fig. 10).

Discussion

Species composition and richness were differentially influenced by disturbance depending on metacommunity heterogeneity, local condition, dispersal, and species ecology (e.g. littoral and pelagic). In disturbed communities, dispersal within heterogeneous metacommunities mitigated the decline in pelagic species richness, allowing them to support more pelagic species than isolated disturbed communities and communities from the homogeneous disturbed metacommunity. In undisturbed communities, dispersal in heterogeneous metacommunities increased the densities of littoral species, making them compositionally distinct from isolated undisturbed communities and communities from the homogeneous undisturbed metacommunity. Heterogeneous metacommunities maintained the pelagic gamma richness of the homogeneous undisturbed metacommunity, mitigating the negative effects that disturbance has on pelagic species. Metacommunity species composition followed the disturbance intensity gradient in which littoral species increased in abundance with disturbance intensity. After the zebra mussels were removed, the disturbed and

undisturbed communities became more similar compositionally within and between metacommunity treatments as algal resources rebounded (Fig. 9). However, the differential effects that dispersal had on disturbed and undisturbed communities became less intuitive in the heterogeneous metacommunities and may have been influenced by stochastic processes or changes in resource quality, indicating that dispersal within heterogeneous metacommunities does not guarantee post-disturbance recovery.

Effects of disturbance intensity on species composition and alpha richness

The localized disturbance caused by the zebra mussels resulted in the decrease of algae (Fig. 2), the decline and possible extinctions of several pelagic zooplankton species (Fig. 3; Table 2), and the increase of littoral zooplankton densities (Fig. 5a). These findings are consistent with other studies that have measured the ecological impacts of zebra mussel invasions on phyto- and zooplankton communities in experimental mesocosms (Dzialowski 2013; Sinclair et al. 2015). This trend persisted not only in the isolated disturbed communities, but also in the homogeneous disturbed metacommunity. However, our hypothesis that the alpha richness of the disturbed patches would decrease with increasing metacommunity disturbance was not supported. When the disturbed communities were part of a heterogeneous metacommunity in which undisturbed communities were present, the adverse local effects that the zebra mussels had on pelagic alpha richness were mitigated so that the disturbed patches supported the same species richness as the undisturbed patches. Numerous studies have also demonstrated the ability of the regional species pool to mitigate species loss or increase species richness of low-quality or disturbed habitat patches through dispersal (Shurin 2001; Davies et al. 2009;

Strecker and Arnott 2010; Thompson and Shurin 2012; Sinclair et al. 2015; Shackleford et al. 2017; Shackleford et al. 2018). Dispersal from the regional species pool may even be more important in maintaining biodiversity in metacommunities that contain low-quality or deteriorating habitat patches than those with high-quality habitats (Chisholm et al. 2010) as low-quality habitats may rely on source-sink dynamics to maintain local populations as I found in the disturbed patches from heterogeneous metacommunities.

I hypothesized that the alpha richness of the undisturbed communities would increase as the regional disturbance intensity gradient decreased, however, the alpha richness of the undisturbed communities were the same across all of the metacommunity treatments and the isolated treatments. The lack of an effect on the diversity of the undisturbed communities was probably due to the initial low regional richness of the metacommunities and the lack of compositional differences at the start of the experiment (Grainger and Gilbert 2016; Resetarits et al. 2018). The study was also of short-duration, which reduced the chances of local stochastic extinctions from taking place at a significant rate.

While the alpha richness of the undisturbed communities did not differ across treatments, the undisturbed communities from heterogeneous metacommunities exhibited distinct species compositions relative to those from the homogeneous undisturbed metacommunity and the isolated communities. Habitat patches can differentially influence each other through dispersal (Davies et al. 2009; Chisholm et al. 2010). For example, Davies et al. (2009) conducted a microcosm study using protists to test the effects of metacommunity resource heterogeneity on protist biodiversity and composition. The habitat types consisted of leaf, wheat, and a mixture of leaf and wheat

as food resources. They found that the wheat habitat patches had a stronger environmental filter than the leaf communities and that the presence of wheat communities had an effect on the leaf communities, but the leaf communities did not have any effect on the wheat communities. I found similar results in our study in which the disturbed communities had a stronger environmental filter than the undisturbed communities, and that the species composition of the undisturbed communities were strongly influenced by the disturbed communities (Fig. 5b), which supported our hypothesis. It seems contradictory that the undisturbed communities promoted higher alpha richness in disturbed communities without changing the composition of the disturbed communities; however, I believe that the high densities of littoral species in the disturbed communities overshadowed any changes that may have occurred in pelagic species richness and composition.

Effects of disturbance intensity on metacommunity composition and gamma richness

I hypothesized that gamma richness would increase in the heterogeneous metacommunities, because the two habitat types would support different species; however, our hypothesis was not supported as gamma richness did not differ between the heterogeneous metacommunities and the homogeneous undisturbed metacommunity (Fig. 4a). Rather than gamma richness being driven by beta diversity, here it was driven by alpha richness. Compositionally, the metacommunities followed the disturbance intensity gradient as I hypothesized. However, due to the presence of pelagic species, the heterogeneous metacommunities were more similar to the homogeneous undisturbed metacommunity than to the homogeneous disturbed metacommunity. It is important to

note that even though Treatment 2 was only one disturbed community away from becoming a fully disturbed metacommunity, it supported significantly more species than the fully disturbed metacommunity as well as a compositionally distinct metacommunity. The presence of a single undisturbed community prevented the degradation of an entire metacommunity, which from a management standpoint is a positive in that a highly-disturbed landscape may still maintain its original biodiversity. However, this is also worrisome, considering the rate at which disturbances can spread across landscapes, particularly in the form of invasive species (Beisner et al. 2003). If the effects of the disturbance on the local patches are relatively invisible as they were in our study, there will be no indicators to warn of the biodiversity decline that will follow when all of the patches within the metacommunity become disturbed. Also, the risk of local extinctions increases as suitable habitat patches become more isolated (Allouche et al. 2012), so while a single undisturbed patch in this experiment was able to mitigate species loss, species richness is likely to decline over time from stochastic extinctions.

Post-disturbance recovery

Removing the zebra mussels had diverse and non-intuitive effects on the species richness and community compositions of disturbed and undisturbed communities across the disturbance intensity gradient, particularly in the heterogeneous metacommunities. Algal abundance quickly rebounded in the disturbed mesocosms after the zebra mussels were removed, becoming indistinguishable from the undisturbed mesocosms by the end of the experiment (Fig. 2). I hypothesized that this rebound in algal resources would encourage increases in alpha richness in the formerly disturbed communities, making

them more similar to the undisturbed communities; however, as discussed above, the alpha richness of disturbed communities from heterogeneous metacommunities was already equal to that of the undisturbed communities prior to the zebra mussels being removed. Instead, I found that the pelagic alpha richness of disturbed communities from the heterogeneous metacommunities declined after the zebra mussels were removed, resembling those from the fully-disturbed metacommunity and isolated disturbed communities (Fig. 7a; Table 2; Table 4). Pelagic species richness had already significantly declined early in the experiment (Fig. 3a), so it is possible that the algal resources or mesocosm environments were not conducive to their long-term success. Since I only measured algae using relative fluorescence, I am unaware of how the phytoplankton communities changed over the duration of the experiment. Therefore, it is possible that even though phytoplankton abundance increased, the phytoplankton communities may have changed and were not suitable for pelagic cladoceran consumption. However, Treatments 0, 1, and 2 continued to support significantly higher pelagic gamma richness than Treatment 3 (Fig.8a; Table 5), demonstrating the importance of maintaining undisturbed or species-rich habitats within a metacommunity to maintain regional biodiversity and that a fully-disturbed metacommunity can have irreversible negative effects on regional biodiversity.

The community and metacommunity species compositions changed in unexpected ways after the zebra mussels were removed. I hypothesized that the disturbed communities from the heterogeneous metacommunities would become compositionally more similar to the undisturbed communities and that the heterogeneous metacommunities would become compositionally similar to the undisturbed

metacommunity, Treatment 0; however, our results did not support these hypotheses. The communities and metacommunities from Treatment 1 and 2 exhibited different compositions that did not correspond with the disturbance intensity gradient, such that Treatment 1 was more similar to Treatment 3, and Treatment 2 was more similar to Treatment 0 (Fig. 9). This pattern may be due to stochastic processes, as population densities can vary between environmentally similar habitats (Forbes and Chase 2002). *Ceriodaphnia* was very abundant in Treatments 0 and 2 but became regionally extinct or uncommon in replicates from Treatment 1. It is possible that some of the communities were starting to deteriorate during the last half of the experiment, whether it be from poor food quality and/or population crashes. Zooplankton communities in natural systems frequently change in composition and abundance overtime due to changes in algal resources, temperature, and predation (Hoffmeyer 2004; Nicolle et al 2011; Shaffer et al 2017;). These mesocosms were also relatively species-poor compared to natural systems, which is common of metacommunity mesocosm experiments (Forbes and Chase 2002; Howeth Leibold 2010; Resetarits et al. 2018). Under such species-poor conditions the loss or gain and relative population size of a single species can have large implications for community composition and richness within and between replicates and treatments.

Implications for Metacommunity Mesocosms Studies

In my study, the regional species pool was dependent upon the communities of the local patches, therefore, the regional species pool's ability to influence the local communities was constrained by the conditions of the local communities. This feedback between the local and regional species pools is the core of metacommunity theory. There is a great

deal of literature demonstrating how regional species pools can mitigate the impacts of a disturbance or other local processes on species richness and composition and aid in post-disturbance recovery (Shurin 2001; Cramer et al. 2008; Strecker and Arnott 2010; Thompson and Shurin 2012; Sinclair et al. 2015). However, few studies have utilized dependent regional species pools to study how the local patch condition(s) feed back into the regional species pool and influence other patches (Forbes and Chase 2002; Chisholm et al. 2010; Pedruski and Arnott 2011; Woods et al. 2016). I define a dependent regional species pool as that which is formed from the local experimental units rather than collected from various natural habitat patches that are independent of the experiment. This is alarming, because in nature, regional species pools are dependent of the local communities, so their ability to mitigate disturbances and other local ecological filters are going to be contingent upon the condition of the communities that make up the metacommunity. With that said, there are trade-offs between the two experimental methodologies and the use of independent and dependent regional species pools is going to depend on the question(s) being asked.

Strecker and Arnott (2010) conducted a similar study to mine using the invasive predatory cladoceran, *Bythotrephes longimanus*, to introduce a local disturbance. However, instead of creating a dependent regional species pool as I did, they used an independent regional species pool to determine how dispersal aided in post-invasion recovery. By the end of their experiment, the post-invaded patches resembled the control patches that had never been invaded (Strecker and Arnott 2010). Since I used a dependent regional species pool, which was less species rich than Strecker and Arnott (2010), the results of our post-disturbance phase were less clear. However, our study demonstrates

that the regional species pool is only beneficial if the landscape still contains undisturbed habitat, which cannot be inferred from studies that use independent regional species pools.

Conclusion

Heterogeneity is representative of many different patterns, gradients, and conditions that will have different implications on local and regional dynamics. Our study is a case of biological heterogeneity, where the competitive exclusion of pelagic zooplankton and facilitation of littoral zooplankton by zebra mussels drove most of the differences between communities. Other types of disturbances, particularly those that cause changes in the abiotic environment are likely to produce different results (Pedruski and Arnott 2011; Chapter 3). In our study, the zebra mussels were not directly lethal to the pelagic zooplankton, therefore, they were able to persist in the disturbed mesocosms through source-sink dynamics. However, abiotic environmental gradients can be lethal to some species, preventing any type of source-sink dynamics from taking place. Thus, heterogeneous patterns that focus on biotic interactions are likely to promote different dynamics than abiotic-driven heterogeneity. Overall, I found that dispersal within heterogeneous metacommunities can mitigate species loss caused by local disturbances, and that local disturbances can influence the species composition of undisturbed communities.

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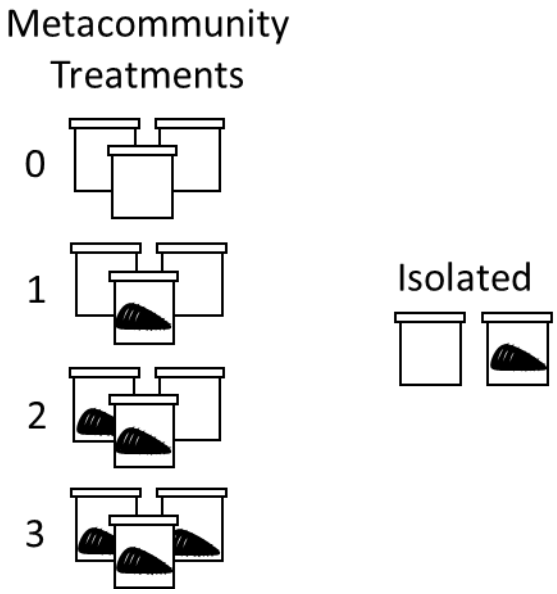

Figure 1. Visual representation of the mesocosm experiment. Each metacommunity consisted of three mesocosms. The numbers on the left indicate treatment number as well as the number of mesocosms within the metacommunity that contained zebra mussels. Each metacommunity and isolated treatment were replicated in triplicate (not shown).

Table 1. Species list for pelagic and littoral groups derived from Dzialowski (2013) and Sinclair et al (2015).

Pelagic Species	Littoral Species
Daphnia parvula	*Scapholeberis mucronata*
Ceriodaphnia lacustris	*Chydorus sphaericus*
Diaphanasoma bergei	*Pleuroxus denticulatus*
Bosmina longirostris	*Alona setulosa*
Moina sp.	*Lathonura rectirostris*

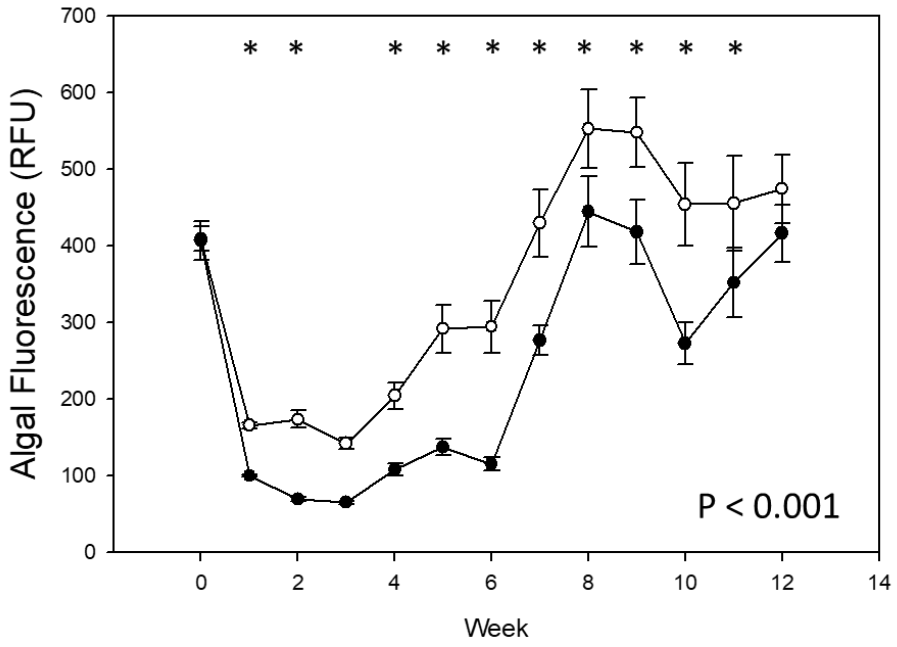

Figure 2. Comparison of algal fluorescence over the course of the experiment between disturbed (closed circles) and undisturbed (open circles) mesocosms. Data were analyzed using a two-way RM ANOVA. Stars (*) represent significant differences ($P < 0.05$) between disturbed and undisturbed mesocosms at specific time points. Error bars are standard error.

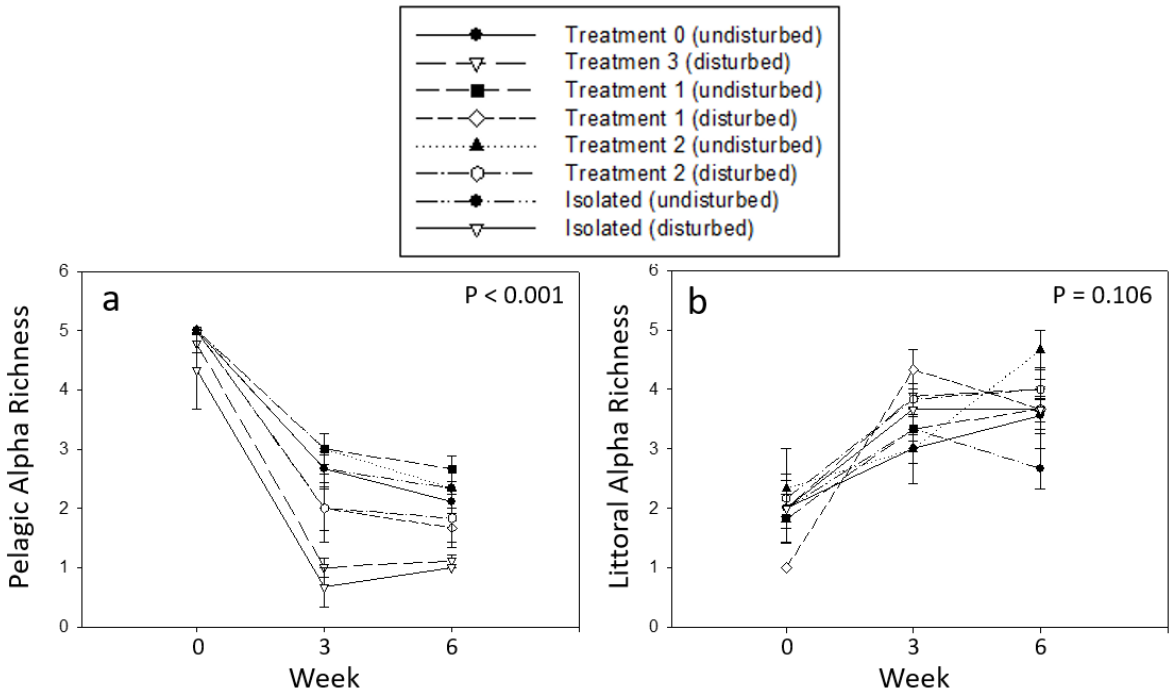

Figure 3. Changes in alpha richness of disturbed and undisturbed communities across metacommunity and isolation treatments during the disturbance phase of the experiment. Data were analyzed using a two-way RM ANOVA. P-values are indicative of overall treatment effects. Error bars are standard error. See Table 2 for pairwise interactions between local treatments for pelagic richness.

Table 2. Matrix of pairwise interactions comparing differences in pelagic alpha richness between local treatments using the Tukey test *post hoc*. Significant differences ($P < 0.05$) are in bold. The numbers preceding the disturbed and undisturbed designations represent the metacommunity treatment.

	1:undisturbed	1:disturbed	2:undisturbed	2:disturbed	3:disturbed	isolated:undisturbed	isolated:disturbed
0:undisturbed	0.491	0.506	0.972	0.414	**<0.001**	1	**<0.001**
1:undisturbed		**0.036**	0.999	**0.013**	**<0.001**	0.947	**<0.001**
1:disturbed			0.259	1	0.056	0.531	**0.009**
2:undisturbed				0.218	**<0.001**	1	**<0.001**
2:disturbed					**0.002**	0.518	**<0.001**
3:disturbed						**<0.001**	0.753
isolated:undisturbed							**<0.001**

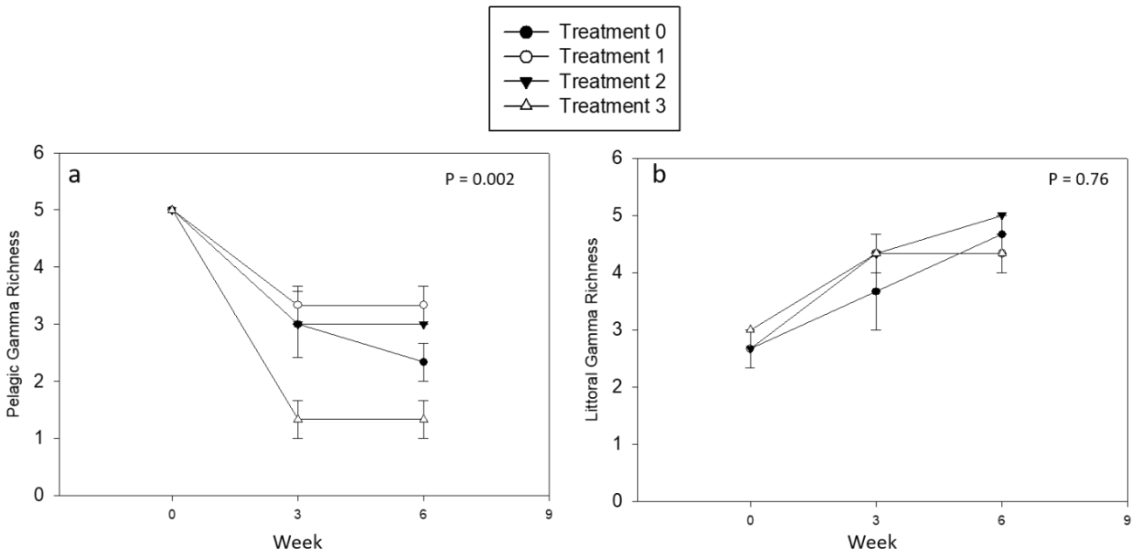

Figure 4. Changes in gamma richness across metacommunity treatments during the disturbance phase of the experiment. Data were analyzed using a two-way RM ANOVA. P-values are indicative of overall treatment effects. Error bars are standard error. See Table 3 for pairwise interactions between local treatments for pelagic

Table 3. Matrix of pairwise interactions comparing differences in pelagic gamma richness between metacommunity treatments using the Tukey test *post hoc*. Significant differences ($P < 0.05$) are in bold.

	Treatment 1	Treatment 2	Treatment 3
Treatment 0	0.306	0.784	**0.023**
Treatment 1		0.784	**0.002**
Treatment 2			**0.007**

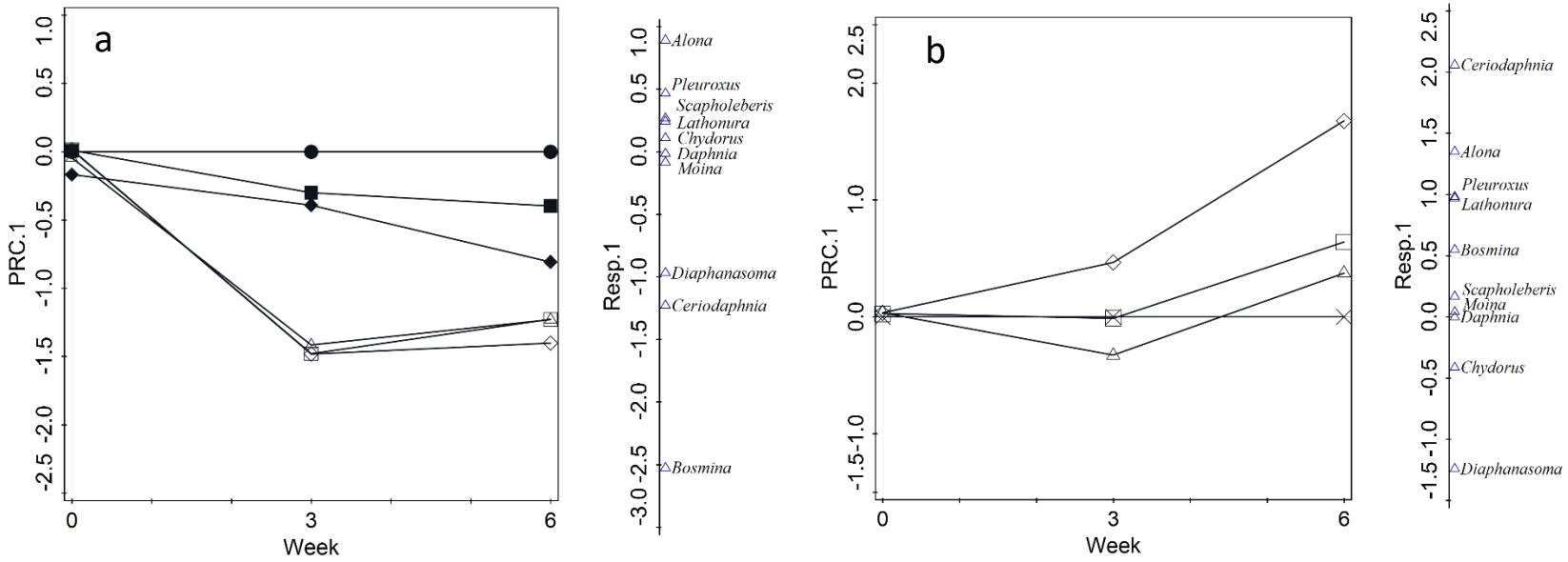

Figure 5. Principal response curves comparing community compositions from the disturbance phase. A) PRC axis 1 (35.9% variance explained, P = 0.002) showing trends in species composition between disturbed (solid shapes) and undisturbed (empty shapes) communities within and between metacommunity treatments with the disturbed communities from Treatment 3 used as the baseline. B) PRC axis 1 (13.05% variance explained, P = 0.05) showing trends in species composition between undisturbed communities across metacommunity and isolation treatments with the undisturbed, isolated treatment as the baseline. Symbols: Treatment 0 (triangle), Treatment 1 (square), Treatment 2 (diamond), Treatment 3 (circle), and Isolated treatments (cross).

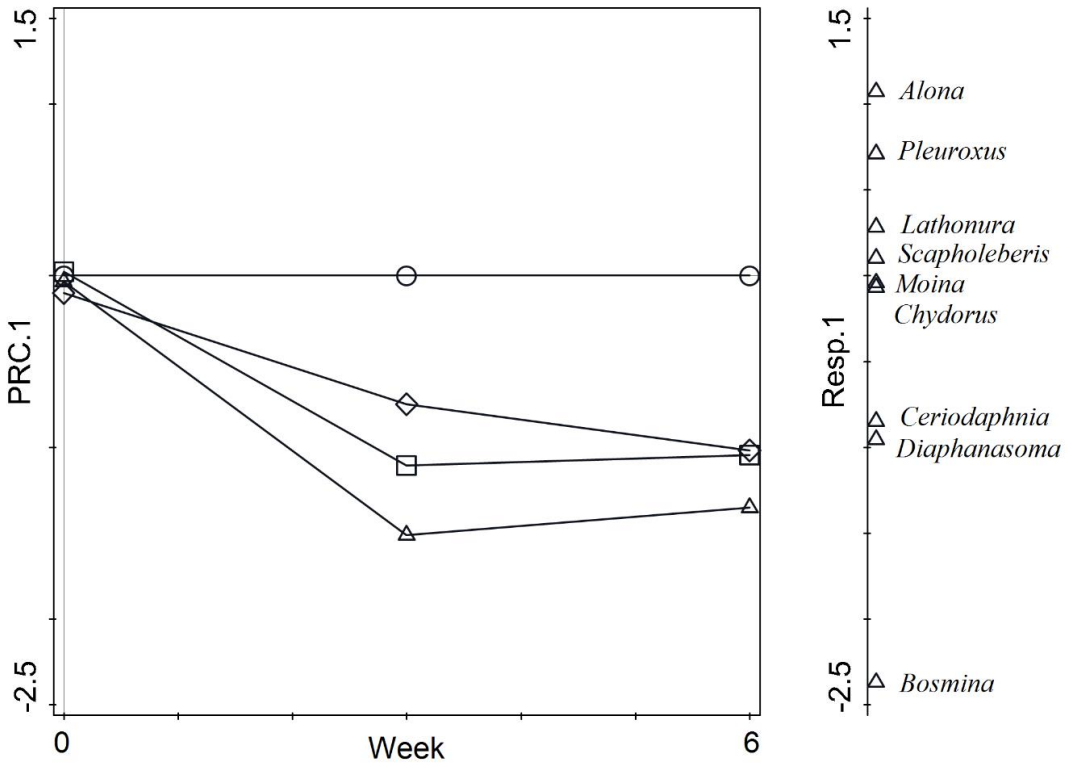

Figure 6. Principal response curves comparing species composition between metacommunity treatments from the disturbance phase, using Treatment 3 as the baseline. PRC axis 1 (24.6% variance explained, P = 0.002) Symbols: Treatment 0 (triangle), Treatment 1 (square), Treatment 2 (diamond), and Treatment 3 (circle).

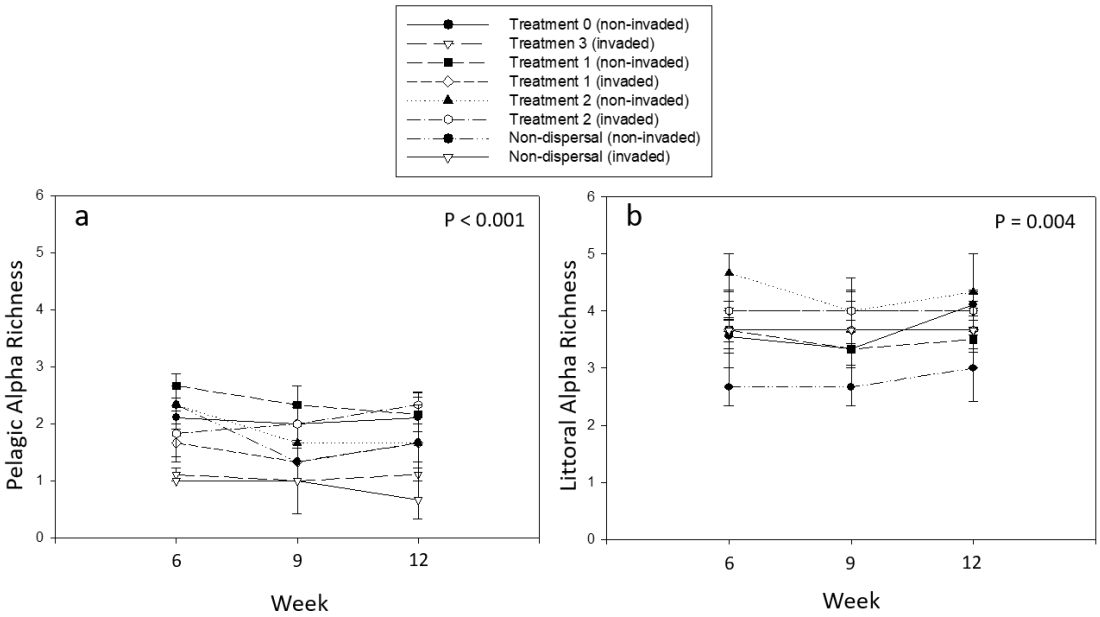

Figure 7. Changes in alpha richness of disturbed and undisturbed communities across metacommunity and isolation treatments during the post-disturbance phase of the experiment. Data were analyzed using a two-way RM ANOVA. P-values are indicative of overall treatment effects. Error bars are standard error. See Table 4 for pairwise interactions between local treatments for pelagic and littoral richness.

Table 4. Matrix of pairwise interactions comparing differences in pelagic alpha richness between local treatments and littoral alpha richness between local treatments using the Tukey test *post hoc*. Significant differences ($P < 0.05$) are in bold. The numbers preceding the disturbed and undisturbed designations represent the metacommunity treatment.

	1:undisturbed	1:disturbed	2:undisturbed	2:disturbed	3:disturbed	isolated:undisturbed	isolated:disturbed
Pelagic							
0:undisturbed	0.939	0.801	1	1	**0.005**	0.998	**0.030**
1:undisturbed		0.335	0.892	0.960	**<0.001**	0.706	**0.005**
1:disturbed			0.922	0.962	0.853	1	0.760
2:undisturbed				1	**0.033**	1	0.077
2:disturbed					0.162	1	0.181
3:disturbed						0.472	1
isolated:undisturbed							0.433
Littoral							
0:undisturbed	0.997	0.952	0.443	0.819	0.997	0.097	0.819
1:undisturbed		1	0.213	0.559	1	0.351	0.987
1:disturbed			0.194	0.438	0.999	0.804	1
2:undisturbed				1	0.156	**0.003**	0.096
2:disturbed					0.525	**0.020**	0.276
3:disturbed						0.254	0.975
isolated:undisturbed							0.927

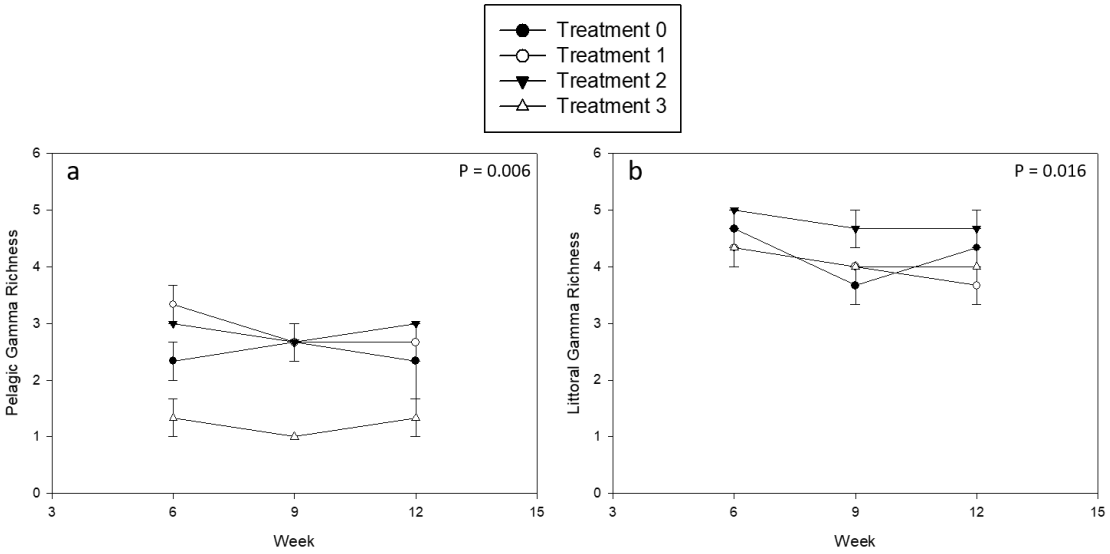

Figure 8. Changes in gamma richness across metacommunity treatments during the post-disturbance phase of the experiment. Data were analyzed using a two-way RM ANOVA. P-values are indicative of overall treatment effects. Error bars are standard error. See Table 5 for pairwise interactions between local treatments for pelagic and littoral richness.

Table 5. Matrix of pairwise interactions comparing differences in pelagic gamma richness between metacommunity treatments and littoral gamma richness between metacommunity treatments, using the Tukey test *post hoc*. Significant differences ($P < 0.05$) are in bold.

	Treatment 1	Treatment 2	Treatment 3
Pelagic			
Treatment 0	0.640	0.640	**0.043**
Treatment 1		1	**0.009**
Treatment 2			**0.009**
Littoral			
Treatment 0	0.669	0.078	0.936
Treatment 1		**0.016**	0.936
Treatment 2			**0.035**

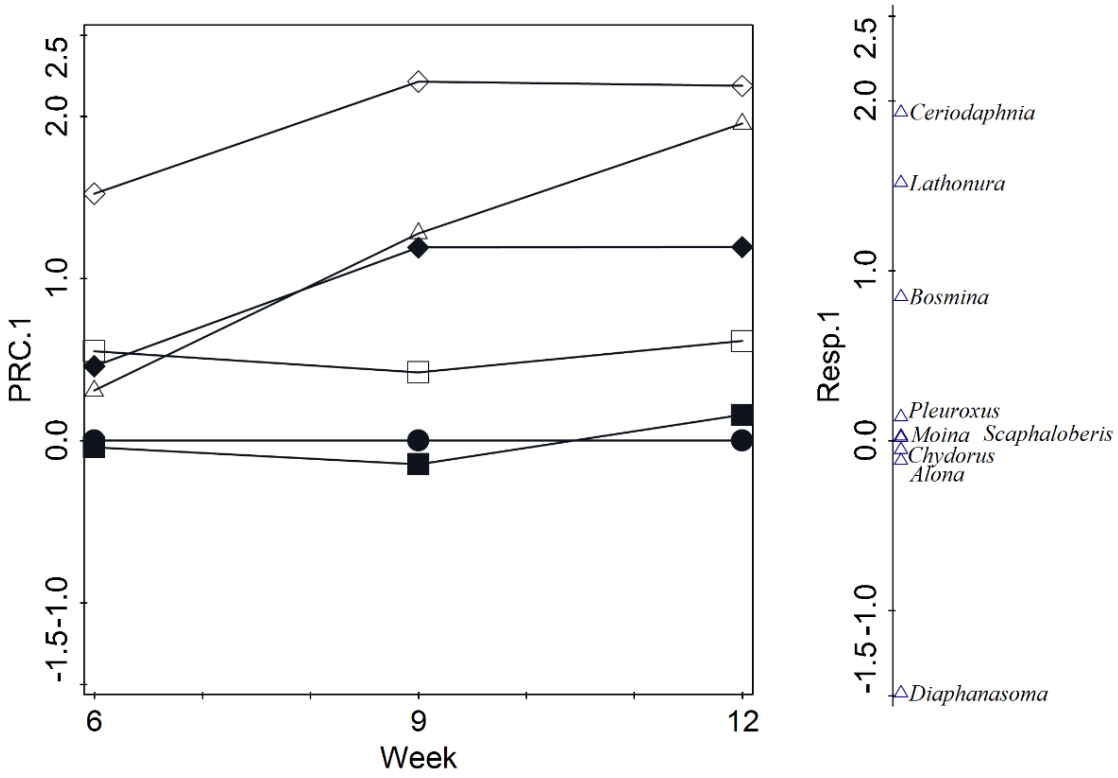

Figure 9. Principal response curves comparing community compositions from the post-disturbance phase. PRC axis 1 (33.4% variance explained, P = 0.001) showing trends in species composition between disturbed (solid shapes) and undisturbed (empty shapes) communities within and between metacommunity treatments with the disturbed communities from Treatment 3 used as the baseline. Symbols: Treatment 0 (triangle), Treatment 1 (square), Treatment 2 (diamond), and Treatment 3 (circle).

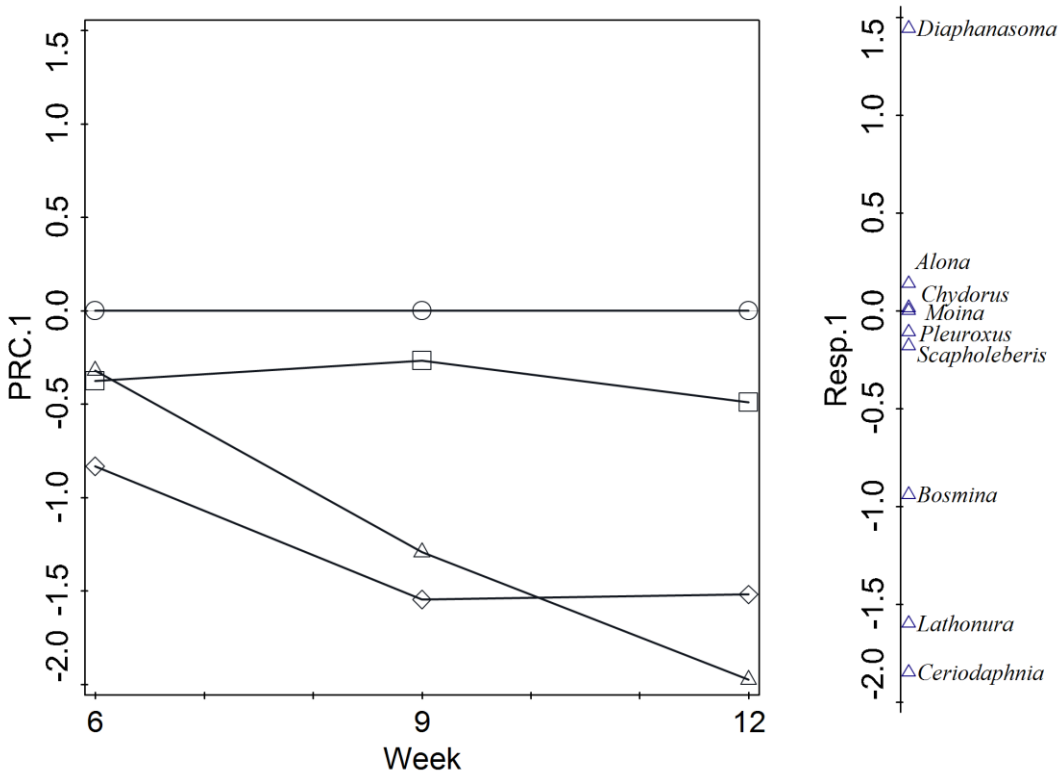

Figure 10. Principal response curves comparing species composition between metacommunity treatments from the post-disturbance phase, using Treatment 3 as the baseline. PRC axis 1 (27.3% variance explained, P = 0.001) Symbols: Treatment 0 (triangle), Treatment 1 (square), Treatment 2 (diamond), and Treatment 3 (circle).

VITA

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Doctor of Philosophy

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